TECHNOLOGY DESCRIPTION

Magnetofection™ technology

Magnetic Plates & Starting Kits

i-MICST™ Technology

Lipofection technology

Polymer-Based Transfection

CRISPR/CAS9 genome editing Transfection reagents

MAGNETOFECTION™ TECHNOLOGY

Invented by Dr. Christian Plank, co-founder of OZ Biosciences, Magnetofection™ is a novel, simple and highly efficient transfection method.
Inspired by the validated and recognized magnetic drug targeting technology, this original method is a revolution for transfection and infection.
In essence, the idea was to unite the advantages of the popular biochemical (cationic lipids or polymers) and physical (electroporation, gene gun) transfection methods in one system while excluding their inconveniences (low efficiency, toxicity, difficulty to handle). It is the unique technology suitable for viral and non viral gene delivery applications.

PRINCIPLE

Magnetofection™ principle is to associate nucleic acids, transfection reagents or virus with specific cationic magnetic nanoparticles.
The resulting molecular complexes are then concentrated and transported into cells supported by an appropriate magnetic field.
In this way, the exploitation of a magnetic force exerted upon gene vectors allows a very rapid concentration of the entire applied vector dose on cells, so that 100% of the cells get in contact with a significant vector dose, and promotes cellular uptake.

HOW DOES IT WORK?

The magnetic nanoparticles are made of iron oxyde, which is fully biodegradable, coated with specific proprietary cationic molecules varying upon applications. Their association with the gene vectors (DNA, siRNA, ODN, virus, etc.) is achieved by salt-induced colloidal aggregation and electrostatic interaction. The magnetic particles are then concentrated on cells by the influence of an external magnetic field generated by a specific magnetic plate. The cellular uptake of the genetic material is accomplished by endocytosis and pinocytosis, two natural biological processes. Consequently, membrane architecture & structure stay intact in contrast to other physical transfection methods that damage, create hole or electroshock the cell membranes. The nucleic acids are then released into the cytoplasm by different mechanisms depending upon the formulation used.
First is the proton sponge effect caused by **cationic polymers** coated on the nanoparticles that promotes endosome osmotic swelling, disruption of the endosomal membrane and intracellular release of DNA.
Second is the destabilization of the endosome by **cationic lipids** coated on the particles that release the nucleic acid into cells by flip-flop of cell negative lipids and charged neutralization.
Third one is the **usual viral mechanism** when virus is used.

BIODISTRIBUTION OF MAGNETIC NANOPARTICLES

The biodegradable cationic magnetic nanoparticles are not toxic at the recommended doses and even higher. Gene vectors/magnetic nanoparticles complexes are internalized into cells after 10-15 minutes i.e. much faster than any other transfection method.
After 24, 48 or 72 hours, most of the particles are localized in the cytoplasm, in vacuoles (membranes surrounded structure into cells) and occasionally in the nucleus. In addition, magnetic nanoparticles do not influence cell function.

WHAT ARE THE APPLICATIONS?

Magnetofection™ is the only versatile and universal technology adapted to all types of nucleic acids (DNA, siRNA, dsRNA, shRNA, mRNA, ODN...), non viral transfection systems (transfection reagents) and viruses. Consequently, several optimized reagents have been designed according to defined applications:

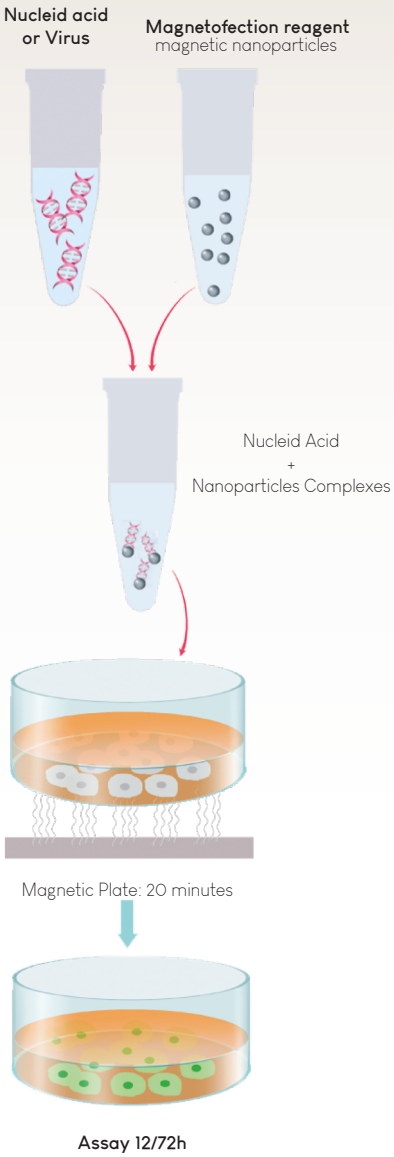
PolyMag/PolyMag Neo: for all nucleic acids transfection
NeuroMag: for neurons transfection
CombiMag: for enhancing all transfection reagents efficiency (cf Magentofectamine)
SilenceMag: for siRNA applications
ViroMag: for enhancing viral transduction efficiency
ViroMag R/L: for Lentivirus and Retrovirus transduction
AdenoMag: for Adenovirus and AAV transduction
FluoMag: Fluorescent Magnetofection Reagents
SelfMag: for creating your own magnetic delivery system

Magnetofection™ has been successfully tested on a broad range of cell lines, hard-to-transfect and primary cells. It is perfect for non-dividing or slowly dividing cells, meaning that **the genetic materials can go to the nucleus without cell division**. We have shown that combining magnetic nanoparticles to gene vectors of any kind results in a dramatic increase of uptake of these vectors and high transfection efficiency. It is the only technology suitable for viruses and non-viral nucleic acid delivery applications.

› For **non viral nucleic acid delivery**, it is perfect for **primary and hard-to-transfect adherent cells**

› For **viral applications**, it is ideal for **any cells** including primary cells (adherent and suspension).

Please consult the technical appendix pages 56-60 for the list of cells successfully tested or contact directly our technical support team at: **tech@ozbiosciences.com**



HOW DO I USE MAGNETOFECTION™ REAGENTS?

The protocol is a very straightforward and easy procedure:

1. Dilute nucleic acids or vectors in serum free medium or buffer and add Magnetofection™ reagent.
2. Incubate 20-30 minutes.
3. Add these complexes directly to cells.
4. Apply the magnetic field (place the culture plate on the magnetic plate).
5. Incubate 5-20 minutes, remove the magnetic plate and cultivate cells until assay.

DO I NEED SPECIFIC EQUIPMENTS?

The only requirement for Magnetofection™ is a magnetic plate specifically designed for this application.

The magnetic plate is a one-time buy and completely reusable, so you do not need expensive equipment contrary to approaches such as electroporation or gene gun. Basically, the magnetic field required is produced by specific magnets. Three magnetic plates are available: Super Magnetic Plate, Magnetic Plate with 96 individuals magnets and Mega Magnetic Plate. Their design allows producing a heterogeneous magnetic field that magnetizes the nanoparticles in solution, forms a very strong gradient to attract the nanoparticles and covers all the surface of the plate.
The plate can be washed with ethanol 70% and used within incubators or robots.

MAGNETIC PLATES & STARTING KITS

Magnetofection™ technology requires appropriate magnetic fields that magnetize nanoparticles in solution, forms a very strong gradient to attract the nanoparticles and covers all the surface of the plate. To perform efficient transfection or infection, suitable magnetic nanoparticles formulations and magnetic field, are the only necessity. Therefore, three optimized magnetic plates with improved properties have been especially designed for Magnetofection™. Their special geometry and organization produce a strong magnetic field that is suitable for all cell culture dishes and supports.
All Magnetofection™ starting kits from OZ Biosciences contain a magnetic plate and the reagents appropriate to your needs; it gives you a convenient solution to start your study.

MAGNETIC PLATES

APPLICATIONS

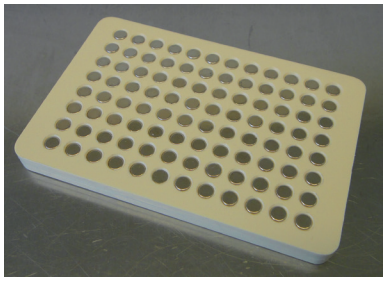
- Suitable for all Magnetofection™ reagents.
- Suitable for all cell culture dishes and supports

Mega Magnetic Plate
To hold 4 culture dishes at one time #MF14000

MAIN FEATURES

- The super Magnetic Plate is suitable for all cell culture support including:
  - 384-, 96-, 48, 24, 12, 6-well plates
  - 35, 60, 90 & 100 mm dishes
  - T-25, T-75 and any other flasks
  - Any other cell culture support (slide, chamber slide, array, roller, etc.)
- The Magnetic Plate with 96-magnets is especially adapted to 96-well culture plates
- The Mega Magnetic Plate can accommodate 4 culture dishes or plates at the same time
- Can be easily cleaned and decontaminated with 70% ethanol
- Can be used within incubators and with robots
- Can be used at room temperature, 37°C, +4°C, etc.
- Compatible with culture plates from most common suppliers
- Magnetic properties, distance between magnets and cells and incubation time have been optimized to efficiently concentrate nucleic acids or virus onto cells and to promote their internalization.
- Solid, completely reusable, it is a one-time buy

Super Magnetic Plate
Convenient for all cell culture support
Catalog number #MF10000



Magnetic Plate with 96 magnets
Adapted to 96-well plates
Catalog number #MF10096

LIPOFECTION TECHNOLOGY

PRINCIPLE

Lipofection is a lipid-based transfection technology which belongs to biochemical methods including also polymers, DEAE dextran and calcium phosphate. Lipofection principle is to associate nucleic acids with cationic lipid formulation. The resulting molecular complexes, known as lipoplexes, are then taken up by the cells. The main advantages of lipofection are its high efficiency, its ability to transfect all types of nucleic acids in a wide range of cell types, its ease of use, reproducibility and low toxicity. In addition this method is suitable for all transfection applications (transient, stable, co-transfection, reverse, sequential or multiple transfections…), high throughput screening assay and has also shown good efficiency in some in vivo models.

HOW DOES IT WORK?

DNA TRANSFECTION MECHANISMS

The lipid-based reagents used for lipofection are generally composed of synthetic cationic lipids that are often mixed with helper lipids such as DOPE(L-a-dioleoylphosphatidyl-ethanolamine) or cholesterol. These lipids mixture assembles in liposomes or micelles with an overall positive charge at physiological pH and are able to form complexes (lipoplexes) with negatively charged nucleic acids through electrostatics interactions. The association of the lipid-based transfection reagent with nucleic acids results in a tight compaction and protection of the nucleic acids and these cationic complexes are mainly internalized by endocytosis. Once inside the cells two mechanisms leading to the nucleic acids release into the cytoplasm have been described. One relies on the endosomes buffering capacity of the polycationic residues (called “proton sponge effect”). The other describes the ability of cellular negatively charged lipids to neutralize the cationic residues of the transfection reagent leading to destabilization of endosomal membranes. Finally, the cellular and molecular events leading to the nuclear uptake of DNA (not required for siRNA) following by gene expression remain highly speculative. However, the significance of cell division on transfection efficiency favours the assumption that nuclear membrane disruption during the mitosis process promote DNA nuclear uptake. Nonetheless, transfections of primary cells (non-dividing) and in vivo are also achievable with lipofection demonstrating that DNA can make its way to the nucleus where gene expression takes place.

TEE-TECHNOLOGY

The cationic lipids (lipoplexes) and polymers (polyplexes) are the most employed non-viral gene delivery systems. The Tee-Technology (Triggered Endosomal Escape) combines and exploits the properties of both entities to achieve extremely efficient nucleic acids delivery into cells. Indeed, this new generation of lipopolyamines contains a lipophilic part, such as lipids, and a charged polyamine moiety, such as cationic polymers. These moieties act in synergy to ensure a tight nucleic acids compaction and protection and a very efficient destabilization of the endosomal membrane which allows the release of large nucleic acids amounts in the cytosol and DNA nuclear uptake. A particular focus on the synthesis of fully biodegradable entities was integrated. In this way, the transfection reagents do not interfere with cellular mechanisms, high cell viability is maintained in every experiment and any potential secondary effects are avoided.

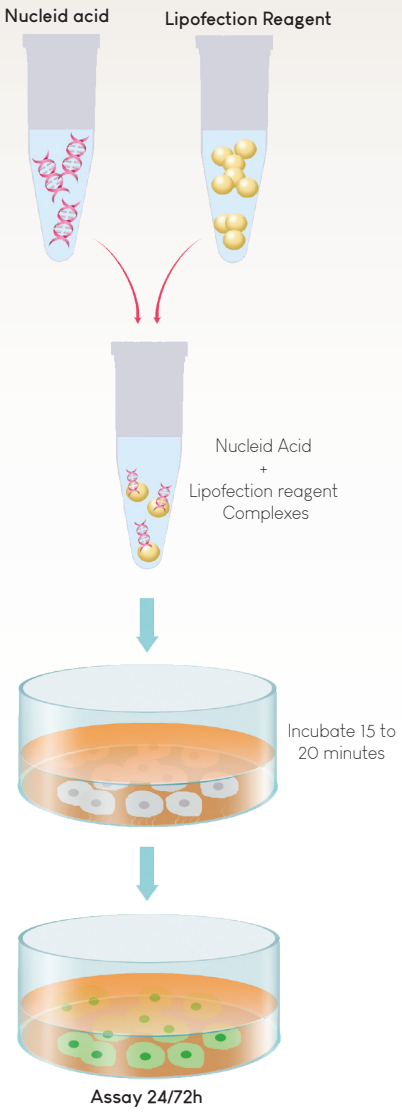
WHAT ARE THE APPLICATIONS?

Transfection efficiency combined with high transgene expression level or high gene silencing and minimized cytotoxicity depends on multiple critical parameters. Those factors include cell type, plasmid DNA characteristics (size, promoter, reporter gene) & purity, siRNA sequence & purity, cell culture conditions (medium with or without serum, cell number, absence of contaminations...), amount of nucleic acids and reagents, transgene assays to name a few. Consequently, transfection reagents need to be specifically designed according to the nucleic acids to be delivered (DNA, siRNA, mRNA, ODN, shRNA etc.) and the cell types used in order to achieve optimal efficiency. In this context, OZ Biosciences has developed several outstanding transfection reagents

- **DreamFect™ Gold:** for all nucleic acids, acheving superior transgene expression level
- **DreamFect™:** for all nucleic acids, for all cells including suspension cell lines
- **Lullaby™:** for siRNA application
- **VeroFect™:** for Vero cells transfection
- **FlyFectin™:** for insect cell transfection
- **EcoTransfect™:** for popular cell lines and routine transfection at low cost

Lipofection method is especially suitable for immortalized cells.

Please consult the technical appendix page 61 for the list of cells successfully tested or contact directly our technical support team at: **tech@ozbiosciences.com**



The major Tee-Technology advantages are:

› Compaction of DNA in nanoparticles efficiently internalized by cells
› Protection of nucleic acids against nucleases degradation
› Efficient membrane destabilization and DNA delivery
› Highly efficient even with low amounts of nucleic acids
› Biodegradability

HOW DO I USE LIPOFECTION REAGENTS?

The protocol is a very straightforward and easy procedure:

1. Prepare the DNA and the Reagent solutions.
2. Mix them together and incubate 20 min.
3. Add to your cells.

i-MICST™ TECHNOLOGY

PRINCIPLE

i-MICST™ Technology (integrated Magnetic Immuno-Cell Sorting and Transfection/ Transduction) is a new platform that allows to genetically modify cells directly on magnetic cell purification columns. This technology combines cell isolation and genetic modification in one simple, efficient and reliable integrated system.
Designed for i-MICST™ Technology, the Viro-MICST™ reagent allows the efficient and specific transduction of target cells directly on magnetic cell-purification columns.

WHY USE VIRO-MICST™?

Viro-MICST™ leads to an increase in the transduction efficiency with low-titer virus preparations compared to regular transduction methods and allows you to reduce cell manipulation steps and save time as well as vector material.

› Isolation and transduction of cells in one reliable integrated system
High and increased transduction efficiency with low MOI
› Acceleration of the transduction process and synchronization of adsorption
› Ideal for sensitive cell types such as primary and stem cells.

HOW DOES IT WORK?

i-MICST™ Technology requires:
› Magnetic cell separation systems (not provided by OZ Biosciences)
› The Viro-MICST™ reagent for capturing virus and infecting cells within the magnetic cell purification column.

Viro-MICST™ is a new specific magnetic nanoparticle formulation evolved from our Magnetofection™ Technology developed in association with MACS® technology* from Miltenyi Biotec.
Viro-MICST™ binds to viruses. As both magnetically labeled target cells and virus-Viro-MICST™ complexes are retained by the magnetic field into the column, the viruses can interact and infect target cells with high efficiency.

The i-MICST™ protocol is depicted as a two-steps process:

1- Pre-enrichment step of magnetically labeled cells on non-modified column(s).
2- Viro-MICST™ procedure. This step allows reaching high purity and simultaneously infecting the target cell population. (cf. fig.1)

RAPID, SIMPLE AND READY-TO-USE
Viro-MICST™ Procedure

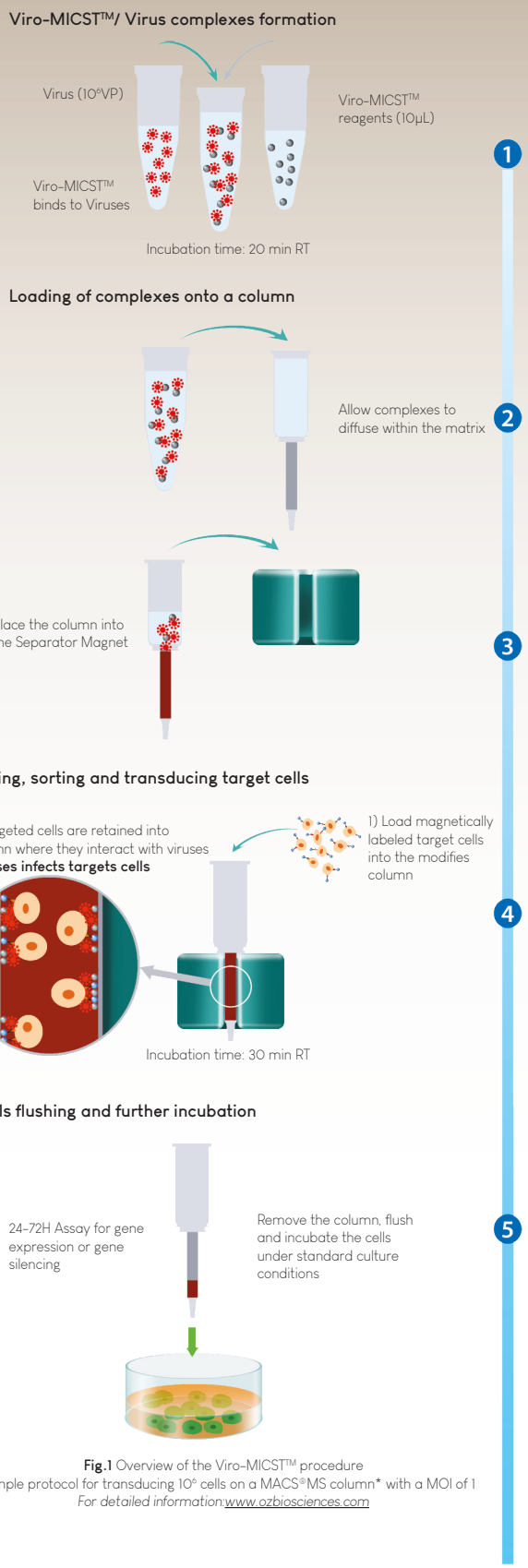


Fig.1 Overview of the Viro-MICST™ procedure
Example protocol for transducing 10^5 cells on a MACS®MS column* with a MOI of 1
For detailed information:www.ozbiosciences.com

**MACS® is a registered trademark owned by Miltenyi Biotec GmbH and the use of MACS® column is proprietary and patented technology. For any further licensed of MACS® system, please contact Miltenyi.*

POLYMER-BASED TRANSFECTION

After the development of Lipofection (lipid-based transfection method) and Magnetofection (magnetic nanoparticles-based transfection method), OZ Biosciences revolutionizes Polyfection with the design and synthesis of a novel patented Cationic Hydroxylated Amphiphilic Multi-block Polymer (CHAMP) which is biocompatible, cleavable, ph responsive and bi-functional.

We created a totally new transfection agent based on the CHAMP technology to mark the separation from what is usually being done with classic transfection methods. This novel bi-functional copolymer is biocompatible, ionizable and pH sensitive. Formed by three moieties, it combines and introduces three synergistic notions:

The concept of "passing through the membranes barriers" due to its charge, pH-sensitive and hydrophobic properties.
The idea of "stealth transfection" where DNA is protected, masked and supported all the way to its nuclear uptake.
The concept of biocompatibility due to biodegradable and cleavable moieties

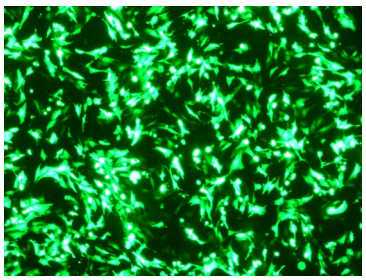
HISTORY

Two main types of delivery vehicles are routinely used for genetic modification of cells: viral and non-viral vectors.

Transport systems have to overcome a series of extracellular and intracellular barriers until the DNA delivery into the cell nucleus. Viruses have evolved in order to bypass each of these checkpoints but despite their efficiency they have to deal with important issues such as immunogenicity, cytotoxicity, safety and target-cell specificity that limit their use.

helix-in transfection reagentInspired by the strategy of some viruses to gain entry into mammalian cells, researchers tried to build synthetic viruses or virus-like particles in order to efficiently and safely transport genetic material to the cell nucleus; the main focus being to mimic viral vectors in terms of performance without encountering their principal pitfalls. The goal was to replicate with the synthetic molecular complexes all the steps used by the viruses to infect mammalian cells.

Non-viral vectors have thus gained increasing attention since several decades as they do not contain any pathogenic proteins and are therefore more likely to be safe. However, synthetic carriers were generally unsatisfactory because they lacked one or several of the necessary functions needed for optimal performance.



Synthetic vectors are materials that electrostatically bind and compact nucleic acid into nanoparticles (tens to several hundreds of nm), protect them from degradation and mediate their entry into cells. Cationic lipids and polymers can be used to complex DNA, creating lipoplexes and polyplexes respectively. The use of cationic lipids for gene delivery was first reported by Felgner in 1987 and lipofection mechanisms are described elsewhere.

Synthetic polymers were also extensively studied principally due to their chemical versatility that "easily" allows generating, modifying and synthesizing linear, branched or dendritic polymeric structures with multiple functions[v].
Cationic polymers play a crucial role for the development of gene transfer agents due to their extraordinarily good potential to condense DNA [vi]. As a result, cationic polymers hold great promises for gene delivery and one of the most used polymers for gene delivery was polyethylenimine (PEI). Numerous drawbacks of PEI have limited its application and many alternatives (polylysine, polyamidoamine, dendrimer, polyallylamine and methacrylate/methacrylamide polymers) have been synthesized gaining ground on efficacy and reducing toxicity without however reaching all the promises.

One of the main issues remains the activation of innate immune response induced by gene delivery system[viii]; trying too hard to mimic virus physiology can result in reaching the dark side. Gene delivery is sensed as a viral or bacterial attack by the cell that answers by disrupting foreign nucleic acid, inactivating transgene expression or undergoing apoptosis. The overall efficiency is thus lowered.

PRINCIPLE

The particularity of this novel CHAMP technology comes from the fact that the bi-functional cationic biopolymer is made up of three moieties, each bearing different characteristics and functions.

- The first part in the vicinity of the polymer binds and condenses DNA to an unprecedented level and contributes to cytosol delivery.
- The second component is a pH responsive and cleavable linker that improves cellular delivery by favoring endosomal membrane destabilization.
- The third moiety with a defined and optimized molecular weight serves as a DNA shield and nuclear uptake facilitator.

The molecular weight and length (unique for each type of polymer) of each moiety are important parameters linked to overall transfection efficiency.

HOW DOES IT WORK?

1- PROTECTION AND SERUM STABILITY

The design of Helix-IN, our new kind of CHAMP polymer allows the positively charged polyplexes to be stable in solution and not to aggregate overtime.

The structure, polyamine composition & grafting density of the CHAMP polymers were finely tuned and optimized to place the polyplexes at the exact interface where solubility is not affected over time. Moreover, hydrophilic groups were ingeniously arranged within the polymer to lower interactions with negatively charged serum proteins (albumin…) for a more efficient gene carrier definition.

Polyplexes remains intact and DNA is protected from degradation…

This positively charged bi-functional polymer presents enhanced DNA-binding properties allowing extent protection of DNA; the positive DNA/polymer charge ratio keeps DNA bound to polymer, playing a key role in protecting nucleic acid from degradation by serum enzyme. We designed this polymer so that no DNA degradation is observed even when incubated in 50% fetal calf serum at 37°C for 24H.

2 - CELLULAR UPTAKE

Cationic complexes bind to cell membrane mainly through electrostatic interactions and most polyplexes are taken up by the cell through endocytosis pathways (macropinocytosis, phagocytosis, endocytosis). One of the most documented routes of endocytosis is mediated by clathrin.
Once endocytosed, complexes are internalized in an early endosome where pH drops from 7.4 (cell surface) to 6.0 (lumen of endosome). The pH will drop to 5 as the endosome progresses to its late phase.

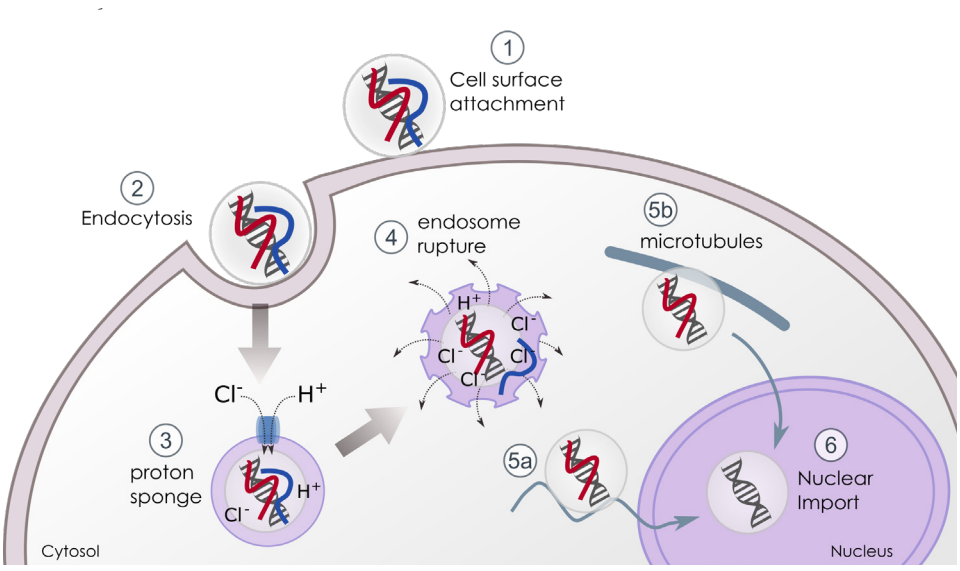


Figure 1. Intracellular trafficking of polyplexes. Excess of polycations at the surface of the polyplex allows attachment to the cell surface (1) and uptake or internalization by the target cell, generally through endocytosis (2). Once into endosome vesicle (3), higher degree of protonation of the proton-sponge polymer causes influx of ions and the pH responsive linker is cleaved, releasing the first polymer unit (blue). The increase of osmotic pressure that leads the vesicle to swell and rupture is favored by the exposition of the hydrophobic domain of the linker (4). The third unit (red) remains bound to the nucleic acid thus lowering the sensing of the DNA by the cell and assisting its nuclear delivery through direct import into the nucleus (5a) or via microtubules trafficking (5b). Once inside the nucleus (6) the DNA is then expressed.

3- ENDOSOMAL ESCAPE & DNA RELEASE

Polyplexes evade endosome and release their cargo into nucleus through the cationic polymer buffering capacities related to the “proton sponge” effectix.

The protonable amines acting as weak base in acidic medium destabilizes pH inside the endosome: once inside the endosomes, specific ATPases generate an influx of protons that are buffered by the polymer.
The massive and continue flow of protons is accompanied with passive entry of chloride ions that results in accumulation of water.
As a consequence, the vesicles swell until endosomal rupture and their content is delivered into the cytosol.

The first polymeric block plays this role.
Moreover the pH responsive and cleavable hydrophobic part adds supplementary features.
Indeed, the linker hidden at physiological pH gets exposed at acidic pH. This leads to its cleavage and to the
hydrophobic zone exposition which promote endosomal membrane fusion/destabilization.

At this stage, several important pitfalls can impair transfection efficiency:

- The capacity of DNA to escape from endosomes is one of the major limitations of the transfection

- It is generally admitted that once delivered into cytosol, DNA must rapidly be imported in the nucleus to avoid cytosolic degradation

- The presence of cell sensors in endosomes (also on cell surface) that can recognize foreign nucleic acids and induce a protective response inhibiting transfection.

4- TRANSPORT & NUCLEAR INTERNALISATION

Once released from endosome, polyplexes have to migrate to the nucleus either via microtubules or through nuclear import machinery. In general, large DNA molecules (>3000bp) and polyplexes remain almost immobile as diffusion is size-dependent into the cytoplasm[ii] and numerous cytosolic nucleases degrade nucleic acids.
Being still complexed to the third moiety of our bi-functional polymer, the smaller positively charged polyplexes can interact with anionic microtubules or motor proteins, or diffuse in a stealth mode until their nuclear uptake. During all these procedures, the DNA is masked and protected from degradation.

The most evident way of nuclear entry for immortalized cells, is during mitosis where redistribution of cellular material occurs and nuclear membrane is disrupted, however, not all the cells follow a proliferative pattern.
Up to now, little is known on nuclear import of polyplex vectors. As soon as DNA has reached the nucleus, it is released from the second part of the polyplexes whom positive charges, molecular mass and grafting design where designed to improve transfection.

WHAT ARE THE APPLICATIONS?

The principal use is DNA transfection for in vitro and *in vivo* applications.

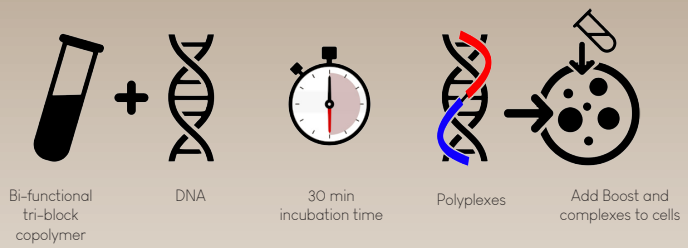
The CHAMP technology increases transfection: more DNA enters the cells and DNA is addressed to the nucleus in a stealth mode without alerting and stressing the cells..This reagent is ideal for immortalized cell lines preferentially adherent such as HEK-293, NIH-3T3, CHO, COS, HeLa, MCF7, MEF, RPE-1; C2C12….

It is perfect for co-transfection of multiple DNA.

In vivo, DNA is condensed and protected into small polyplexes that limit immune responses and are able to navigate through circulatory system until they delivery.

WHAT IS THE PROTOCOL?

The protocol is simple: transfection reagent is directly mix with DNA using ratios 1:1 to 3:1 (1µL per µg DNA to 3µL per µg DNA) depending on the cell type. After 30 min of incubation time, polyplexes and boost are added onto cells. This 30 min incubation time is the cornerstone of the protocol allowing a full compaction and protection of DNA.



During the nanoparticles/DNA complexes self-assembly, it is critical to wait at least 30 minutes to enable the co-polymers and DNA to form stable supramolecular nanoparticles. Due to the multipart nature of the copolymer, the time for forming and stabilizing the complexes is slightly longer than with “simple” polymers where complexes formation occurs more rapidly (10-20 min).

WHAT ARE THE MAIN DIFFERENCES BETWEEN LIPOFECTION AND POLYFECTION?

Lipofection and polyfection (respectively lipid-based and polymer-based transfection) are two methods of transfection using synthetic vectors to deliver nucleic acids into cells. Even if the finalities of the two techniques are the same, some differences still exist orienting the nucleic acid delivery applications to one or the other (refer to table below).

LIPOFECTION AND POLYFECTION (RESPECTIVELY LIPID-BASED AND POLYMER-BASED TRANSFECTION) ARE TWO METHODS OF TRANSFECTION USING SYNTHETIC VECTORS TO DELIVER NUCLEIC ACIDS INTO CELLS.

	LIPOFECTION	POLYFECTION
Structure, properties, mechanism delivery	1.Liposomes, micelles, inversed miceles (amphipatic) – hydrophobic 2.Fusion:destabilization:flip-flop 3.Cytoplasmic release 4. Can be used alone or in presence of colipid 5. Generally based on the sme model hydrophilic head groups, hydrophobic anchor and linker	1. Linear, branched or spherical 2. Water soluble, high charge density 3. Proton-sponge effect 4. Nuclear uptake possible 5. Various designs, grafting composition, lenght; MW...
Strengths	·Versatile:any nucleic acids ·Biodegradable ·Excellent biocompatibility with cellula membrane ·No package size limits ·Short time required for formation of complexes ·Superior bioavailability when complexes are formed	·High DNA condensation/delivery ·Biodegradable ·Low cellular stress ·High structural intergrity and stability over storage ·Low toxicity (low to medium MW polymers) ·Stealth transfection ·Increased stability of polyplexes over time ·No autofluorescence
Weaknesses	·Autolfuorescence ·Low structural integrity ·Can interfere with lipids signaling ·Fast clearance in vivo in systemic circulation ·Mild inflammatory response in vivo ·Not applicable to all cells (primary) ·Nedd of a colipid	·Not applicable to all nucleic acids ·Not good for suspension cells ·HMW polymers showt toxicity ·Not applicable to all cells (primary)

CRISPR/CAS9 GENOME EDITING

"Genome editing" or "Genome engineering" gives the ability to introduce a variety of genetic alterations (deletion, insertion…) into mammalians cells. During the past decade, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were the tools of choice for genome editing technologies until the very recent discovery of CRISPR/Cas9 technology that have revolutionized the field.

Successful CRISPR/Cas9 genome editing can be performed through diverse approaches (plasmids, mRNA, nuclease, viral delivery). Accordingly, efficient nucleic acid delivery (transfection or transduction) represents a critical step for genome editing experiments. With more than 10 years of expertise in the development of transfection reagents, OZ Biosciences offers tailored transfection solutions for CRISPR/Cas9 technology.

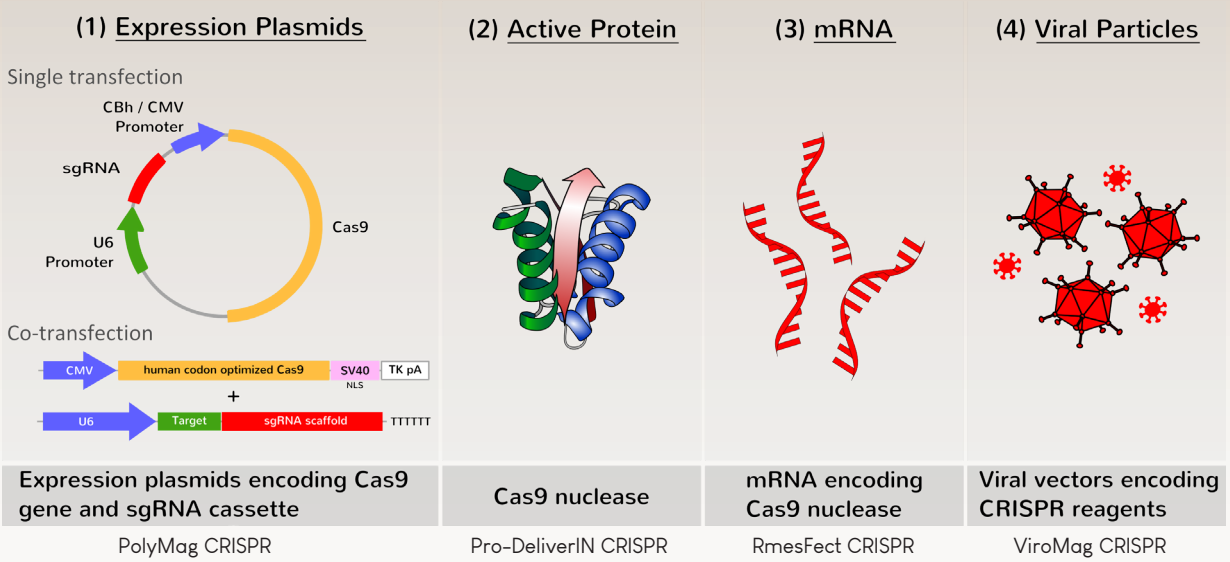


***Figure 1**: Adapted transfection reagents for each CRISPR/Cas9 approach.*
For generation of cellular models, Cas9 and the designed sgRNA (a chimeric RNA containing all essential crRNA and tracRNA components) can be introduced into the target cells. The type II CRISPR/Cas system only needs a single Cas protein that can be expressed into target cells by: (1) plasmid transfection, (2) direct delivery of the active Cas9 endonuclease, (3) transfection of mRNA encoding for Cas9 or (4) by viral vectors transduction.

Transfection Reagents For CRISPR/Cas9

Product Name	Molecul vector	Technology	Application
PolyMag CRISPR	Plasmid DNA	Magnetofection	Primary and hart-to-transfect cells
Pro-deliverIN CRISPR	Protein	Lipofection	All cells
RmesFect CRISPR	mRNA	Lipofection	All cells
ViroMag CRISPR	Virus	Magnetofection	All cells including primary and hart-to-transfect cells

GENOME EDITING WITH CRISPR/CAS9

In 2013, four groups demonstrated that CRISPR/Cas9 associated with guide RNA can be used for gene editing [2-5]. Based on the type II CRISPR/Cas9 mechanism, researchers created a single guide RNA (sgRNA), which is able to bind to a specific dsDNA sequence. This resulted in double strand breaks (DSB) at target site with: (1) a 20-bp sequence matching the protospacer of the guide RNA and (2) a protospacer-adjacent motif (PAM) 3 bp downstream NGG sequence.
CRISPR/Cas9-mediated genome editing thus depends on the generation of DSB and subsequent cellular DNA repair process. The presence of DSB in the DNA generated by CRISPR/Cas9 leads to activation of cellular DNA repair processes, including non-homologous end-joining (NHEJ)-mediated error prone DNA repair and homology-directed repair (HDR)-mediated error-free DNA repair.
Insertions and deletion mutations at target site generated by NHEJ and HDR allow disrupting or abolishing the function of a target gene. Moreover, modifications in this system can also be used to silence gene, insert new exogenous DNA or block RNA transcription.

HOW DOES CRISPR/CAS9 WORK?

CRISPR/Cas9 system originates from bacteria in which it provides acquired immunity against invading foreign DNA via RNA-guided cleavage [1]. Bacteria collect “protospacers”, short segments of foreign DNA (e.g. from bacteriophages) and integrate them into their genome. Sequences from CRISPR genomic loci are then transcribed into short CRISPR RNA (crRNA) that anneal transactivating crRNA (tracrRNAs) to destroy any DNA sequence matching the protospacers. After transcription and processing, crRNA first complexes with Cas9 and tracrRNA and then bind its target sequence onto DNA. Both R-loop forms and DNA strands are cut. crRNA is used as a guide while Cas9 acts as an endonuclease to cleave the DNA.

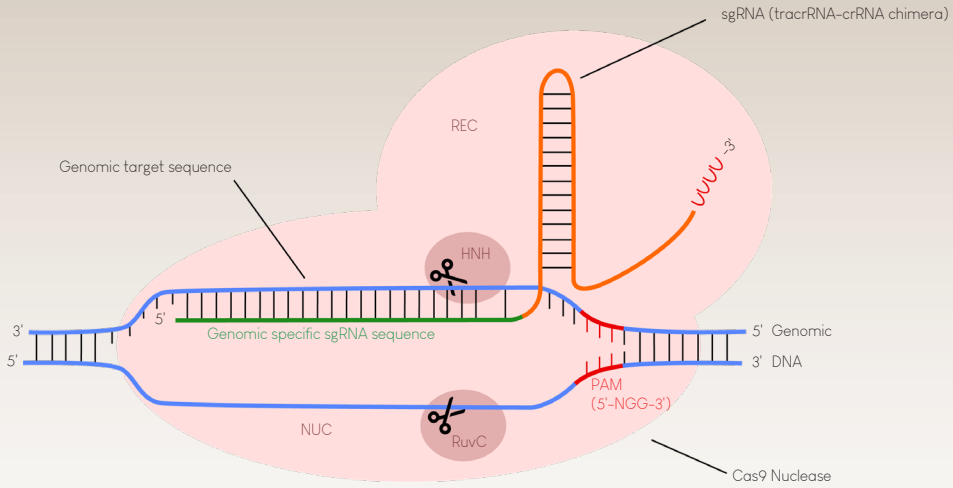


Figure 2: The CRISPR-Cas9 nuclease programmed with sgRNA. Upon binding the sgRNA guide (tracrRNA-crRNA) specifically targets a short DNA sequence-tag (PAM) and unzips DNA complementary to the sgRNA. sgRNA-target DNA heteroduplex, triggering R-loop formation results in a further structural rearrangement: Recognition (REC) and Nuclease lobes (NUC) undergo rotation to fully enclose the DNA target sequence. Two nuclease domains (RuvC, HNH) each nicking one DNA strand, generate a double-strand break. Structurally, REC domain interacts with the sgRNA, while NUC lobe drives interaction with the PAM and target DNA.

VARIOUS CAS9-BASED APPLICATION:

Indel (insertion/deletion) mutations,
Specific sequence insertion or replacement
Large deletion or genomic rearrangement (inversion or translocation)
Fusion to an activation domain :

- Gene Activation
- Other modifications (histone modification, DNA methylation, fluorescent protein)
- Imaging location of genomic locus.

CRISPR/CAS9 ADVANTAGES OVER ZFNS AND TALENS

CRISPR/Cas9 can easily be adapted to any genomic sequence by changing the 20-bp protospacer of the guide RNA; the Cas9 protein component remaining unchanged. This ease of use presents a main advantage over ZFNs and TALENs in generating genome-wide libraries or multiplexing guide RNA into the same cells.

- ZFNs and TALENs are built on protein-guided DNA cleavage that needs complex protein engineering.
- CRISPR/Cas9 only needs a short guide RNA for DNA targeting.
- CRISPR/Cas9 allows using several gRNA with different target sites: simultaneously genomic modifications at multiple independent sites [2].
- Accelerates the generation of transgenic animals with multiple gene mutations [6].

CRISPR/Cas9 system presents a versatile and reliable genome editing tool to facilitate a large variety of genome targeting applications. CRISPR/Cas9 components comprise an endonuclease and a sgRNA that can be delivered into cells under various forms (i.e. plasmid, mRNA, nuclease, virus).

DNA TRANSFECTION

Broad Spectrum

Helix-IN™

Cell Lines

DreamFect™ Gold / DreamFect™ / LipoMag kit

EcoTransfect

Primary Cells

PolyMag / PolyMag Neo

CombiMag / LipoMag kit

Magnetofectamine™ O2

Cell Specific

NeuroMag

Glial-Mag

CosFect / HeLaFect

VeroFect / FlyFectin™

DreamFect Stem

siRNA/mRNA TRANSFECTION

siRNA Transfection Reagents

Lipofection Technology

- Lullaby
- Lullaby Stem

Magnetofection Technology

- SilenceMag

mRNA Transfection Reagents

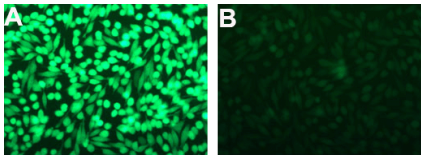
Lipofection Technology

- RmesFect
- RmesFect Stem

Lullaby®- A sweet song for gene silencing

Lullaby is the ideal siRNA transfection reagent for gene silencing. Relying on the TEE-technology (*For more information see page°),* it has been successfully tested on numerous cell lines, reaching up to 90% gene silencing with high reproducibility and a very low toxicity. It protects siRNA from extracellular degradation and has an outstanding ability to destabilize cell membranes. It allows reproducible delivery of important siRNA amounts into the cytosol and high cell viability is maintained in each experiment.

GFP silencing in HeLa cells



GFP-expressing HeLa cells (A) seeded in a 24-well plate were transfected with 1µL Lullaby + 5nM (33.75ng) siRNA (B). GFP-extinction was monitored 72H post-transfection by fluorescence microscopy.

APPLICATIONS

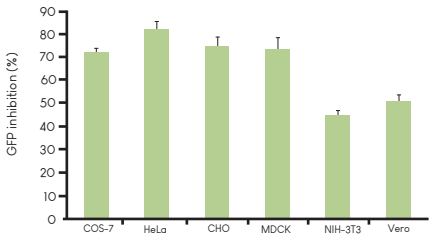
- **Perfect for all gene silencing applications:** siRNA (including endogenous targeting and co-transfections), shRNA, miRNA, dsRNA
- **Suitable for all mammalian cells:** Cell lines, hard-to-transfect & primary cells

RECOMMENDED APPLICATIONS
siRNA transfection of cell lines.
Perfect for High-Throughput Screening

MAIN FEATURES

- **Effective at multiple siRNA concentrations – Minimize off-target effect**
  Lullaby is efficient starting from 0.1nM of siRNA and optimal at 5 to 10nM, avoiding non-specific effects
- **Powerful for all cell types. Up to 90% gene silencing**: 3T6, A549, BHK-21, CHO, COS-7, CV-1, H441, HEK293, HeLa, M1...
- **Flexible & adapted** to all culture conditions Lullaby is antibiotics and serum compatible and works over a broad range of cell confluencies (between 20 to 90%)
- **Versatile & convenient** for all siRNA applications
  Tested on various RNAi targets (GAPDH, GFP, Kinase, LacZ, Lamin, Luciferase...) and with synthetic siRNA & shRNA from different suppliers.
- **Rapid, easy procedure & biodegradable**

GFP silencing in various cell lines with Lullaby



GFP-expressing cells were seeded on a 24-well plate and transfected with 10nM (67.5ng) siRNA associated with 2µL of Lullaby. GFP-extinction was monitored 72H post-transfection by flow cytometry.

TESTIMONIAL

"We initially collated a transfection reagent library of 26 reagents[...] By far, our preferred reagent is Lullaby from OZ Biosciences. We have used this reagent in over 20 cell lines and have found it essential in enabling siRNA screens in hard to transfect cell lines [...], with minimal toxicity".
Shanks Emma.L. *et al* - **Strategic siRNA Screening Approaches to Target Cancer at the Cancer Research UK Beaston Institute, Combinatorial Chemistry & High Throughput Screening.2014**

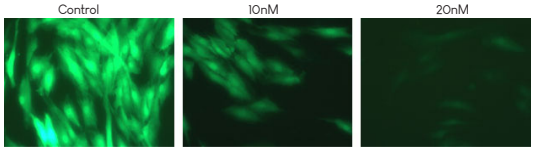
Cat. No.	Product	Number of assays
LL70500	Lullaby 500 µL	Up to 1000
LL71000	Lullaby 1 mL	Up to 2000
LL73000	Lullaby 3x1 mL	Up to 6000

Successfully tested and published !

Browse our citation database online

Lullaby Stem

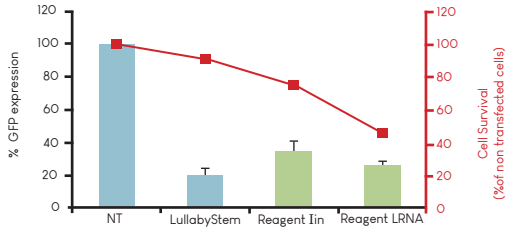
Lullaby Stem siRNA Transfection Reagent is ideal for gene silencing in Stem Cells.



Representative pictures of GFP-stably transduced human AFSC 48H after treatment with Lullaby Stem transfection reagent and increasing doses of siRNA targeting GFP (x400).

RECOMMENDED APPLICATIONS
Gene silencing into stem cells, embryonic stem cells, Multipotent stem cells,iPS

Comparison of Lullaby Stem with other siRNA transfection reagents



MAIN FEATURES

- **Minimized toxicity** due to reagent biodegradability and low siRNA/miRNA amount required
- **Reliable and reproducible** gene knockdown results
- **Serum & Antibiotics compatible**

Cat. No.	Product	Number of assays
LS20500	Lullaby 500 µL	Up to 250
LS21000	Lullaby 1 mL	Up to 500

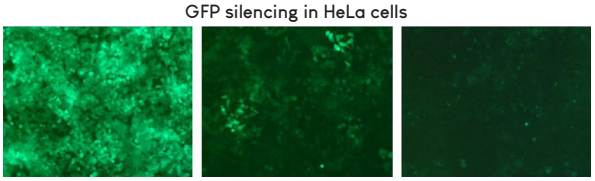
SilenceMag – The bright idea for siRNA delivery

SilenceMag has been developed specifically for siRNA delivery. These magnetic nanoparticles are coated with a unique cationic lipids formulation providing the most efficient siRNA delivery system available. It allows studying gene silencing at very low doses of siRNA thanks to the magnetic field mediated concentration of siRNA onto cells. This reagent is suitable for all siRNA applications and gives reliable and high gene knockdown in numerous cell types.

APPLICATIONS

- **Ideal for gene Silencing**: siRNA, dsRNA, shRNA
- **Suitable for mammalian cells**: Cell lines, primary and hard to transfect cells
- **Perfect for all silencing applications**:
  -Sequential & simultaneous transfections and endogenous gene silencing

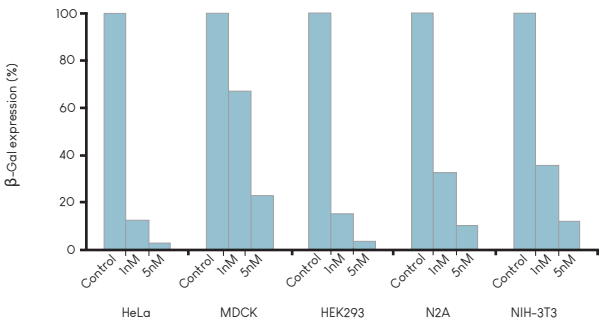
GFP silencing in HeLa cells



GFP-expressing HeLa cells seeded in a 96-well plate were transfected with 0.5 µL of SilenceMag and 4 or 10nM siRNA (corresponding to 10.8 or 27 ng respectively). GFP-extinction was monitored 72H post-transfection by fluorescence microscopy.

RECOMMENDED APPLICATIONS
siRNA transfection of primary and hard-to-transfect adherent cells

LacZ silencing in various cell lines



Various cells were co-transfected in 96-well plates with 100ng of pLacZ plasmid complexed to 0.1µL of PolyMag (#PN30100) and either 1 or 5nM of siRNA associated with SilenceMag.
β-galactosidase expression was monitored after 48H using OZ Biosciences ONPG assay kit (#GO10001).

MAIN FEATURES

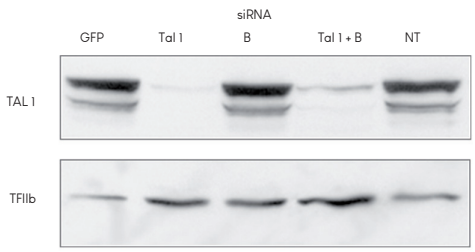
- **High gene silencing efficiency**
  Concentrates and introduces large quantities of siRNA duplexes into cells leading to exceptional knockdown effects
- **Use 10 to 100 times less siRNA**
  Gene silencing can be observed at 0.1 nM and efficiency is optimal at 5 to 10 nM
- **One reagent validated for all siRNA applications**
  Effective for endogenous applications as well as co-transfection
- **Ideal for cell lines & primary cells**
  Primary cells: Airway epithelial, Chondrocyte, Endothelial (PAEC, HUVEC...), Fibroblast, Gastric gland, Epithelial, Keratinocyte, Myofibroblast, etc
  Immortalized cells: 3T6, A549, BEAS-2B, BHK-21, CHO, COS-7, CV-1, H441, HEK293, HeLa, Hep2, Hep3B, HMEC-1, MCF-7, MDCK, N2A, NIH-3T3, U87, Vero, etc.
- **Serum compatible & non toxic**
- **Simple & ready-to-use**
- **Many targeted genes**: GAPDH, GFP, IGFBP, LacZ Lamin, Luciferase, Transcription factors, ROCK, etc.

TESTIMONIAL

"68% of HUVEC were efficiently transfected".
Dou L. *et al* - **J Am Soc Nephrol.2015**

"90% gene silencing in Primary human endothelial colony forming Cells".
Hubert L. *et al* - **J Thromb Haemost.2014**

Tal1 inhibition in primary HUVEC



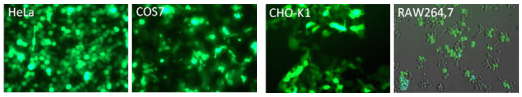
Cat. No.	Product	Number of assays in 96-well plate with 10nM siRNA
SM10200	SilenceMag 200 µL	> 400 assays
SM10500	SilenceMag 500 µL	> 1000 assays
SM11000	SilenceMag 1 mL	> 2000 assays
SM13000	SilenceMag 3x1 mL	> 6000 assays
KC30300	SilenceMag Starting Kit	Contains 1 magnetic plate + 200 µL SilenceMag

This product is also available fluorescently-labelled with TRITC: FluoMag-S (#FS10100)

Magnetofection Technology - This reagent needs to be used with a magnetic plate

RmesFect

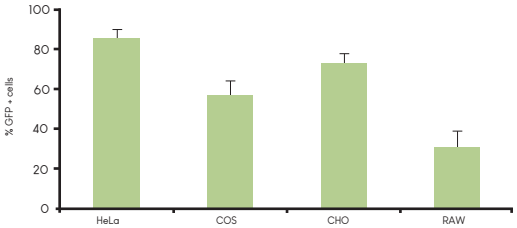
RmesFect™ Transfection Reagent is based on the TEE-technology specifically designed for mRNA transfection with high efficiency and low toxicity.



Several cell lines were transfected with mRNA encoding GFP protein and RmesFect™ transfection reagent. 24H after, transfection efficiency was measured by fluorescence microscopy.

RECOMMENDED APPLICATIONS
Transfection of mRNA in primary cells and cells lines

RmesFect™ transfection efficiency in cell lines



Several cell lines were transfected with 0.5 µg of mRNA encoding GFP protein (ratio 2:1 for RAW,3:1 for COS7 and CHO-K1 and 4:1 for HeLa cells). After 20 min of incubation at room temperature, complexes were added onto the cells in a dropwise manner. 24H after, transfection efficiency was measured by FACS analysis.

MAIN FEATURES

- **Ready-to-use:** no need for additional buffer
- **Low nucleic acid amount:** minimized toxicity
- **Protects mRNA against degradation**
- **Compatible with any culture medium**
- **Serum compatible:** Medium changed not required

Cat. No.	Product	Number of transfections with 1µg of mRNA
RM20500	RmesFect 500µL	125-250
RM21000	RmesFect 1mL	250-500
RM25000	RmesFect 5mL	1250-2500

mRNA transfection provides several advantages over plasmid DNA delivery :

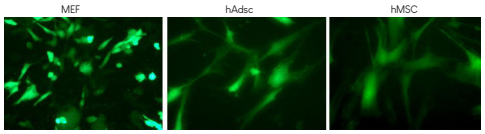
- No need for nuclear uptake mRNA translation into proteins occurs in the cytoplasm.
- Faster protein expression than DNA transfection.
- No genomic integration.
- Protein expression in a total promoter-independent manner.

APPLICATIONS

- **Perfect for all** your mRNA transfection applications:
  - mRNA vaccines/ primary cells transfection
  - Regenerative medicine
  - Cell reprogramming
  - iPs generation
  - Embryonic and multipotent stem cells transfection

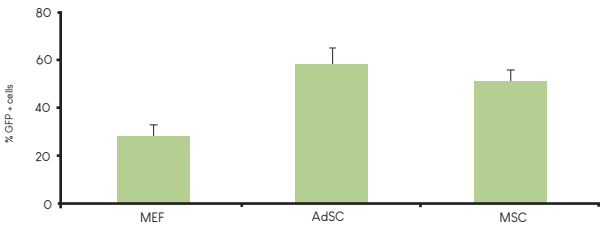
RmesFect Stem

RmesFect™ Stem Transfection Reagent is based on the TEE-technology specifically designed for mRNA transfection in Stem Cells with high efficiency and low toxicity.



Several Stem cells were transfected using RmesFect Stem. 24H after, transfection efficiency was measured by fluorescence microscopy.

RECOMMENDED APPLICATIONS
Transfection of mRNA in stem cells



Several Stem cells were transfected using RmesFect Stem. Complexes were prepared as followed: mRNA encoding GFP protein (0.25 µg for human hMSC and 0.5 µg for MEF and hAdSC) was mixed with RmesFect Stem (ratio 2:1 for hMSC and 4:1 for MEF and hAdSC). After 20 min of incubation at room temperature, the complexes were added onto the cells in a dropwise manner. 24H after, transfection efficiency was measured by FACS analysis.

MAIN FEATURES

- **Ready-to-use:** no need for additional buffer
- **Low nucleic acid amount:** minimized toxicity
- **Protects mRNA against degradation**
- **Compatible with any culture medium**
- **Serum compatible:** Medium changed not required

Cat. No.	Product	Number of transfections with 1µg of mRNA
RS30500	RmesFect Stem 500µL	125-250
RS31000	RmesFect Stem 1mL	250-500
RS35000	RmesFect Stem 5mL	1250-2500

BIOPRODUCTION

1 page techno

Hype 293 / Hype-5

Hype-CHO / Hype-5

3D TRANSFECTION

Transfection in 3D cell culture

3D-Fect™ / si3D-Fect™

3D-FectIN™ / si3D-FectIN™

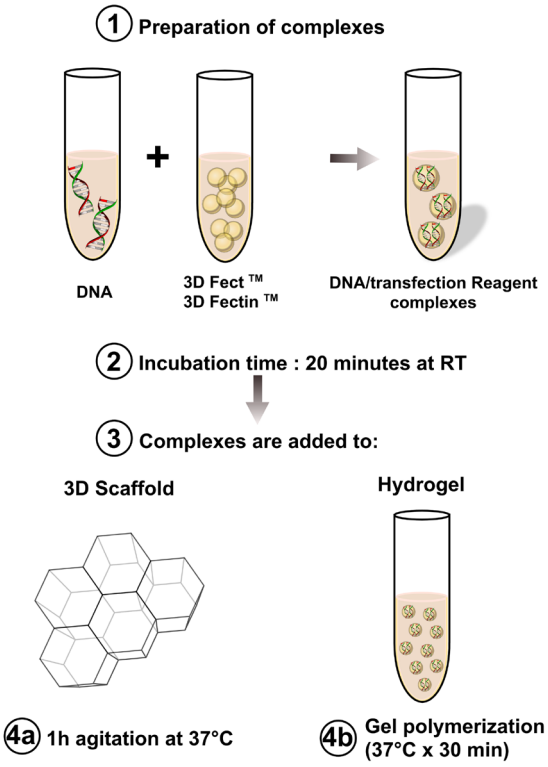
Transfection in 3D cell culture

PRINCIPLE

Three-dimensional (3D) matrices, such as 3D-scaffolds and 3D-hydrogels, work as mechanical platforms for cell attachment and growth. Biomaterials, having a viscoelastic support in constant adaptation to external constraints and responding to numerous physiological stimuli, have been designed to mimic the organic milieu for cells[1].

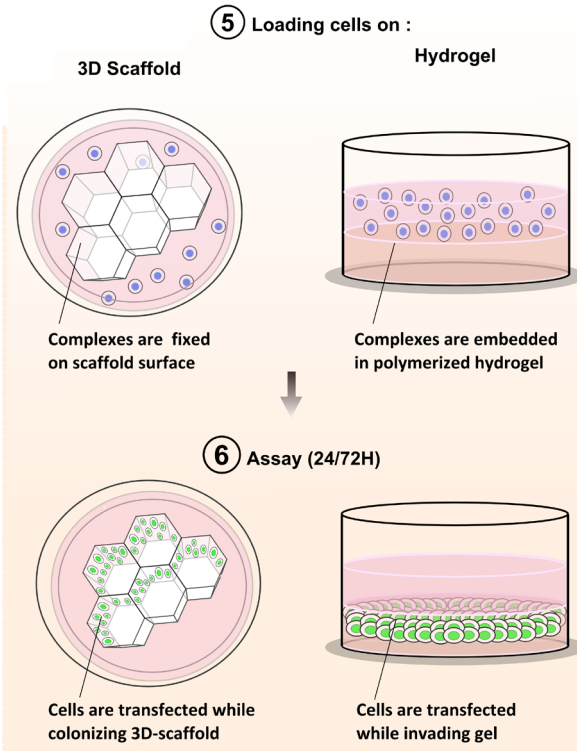
3D matrices allow cultivating cells in vitro in a more natural way. Therefore, 3-D cell cultures assist the cell physiology analysis under conditions that more closely resemble to an in vivo-like environment compared to conventional 2-D culture. Since last decade, it has been proposed that genetically modified cells growing on-, or embedded in 3D matrices could be used as a drug controlled release system[2]. Biomaterials for controlled delivery of plasmid DNA or siRNA can thus provide a fundamental tool to target transgene expression (over express or block) or can offer new perspectives for gene or cell therapy.

3D matrices can be composed by numerous materials (collagen, atelocollagen, polymers, hyaluronic acid, fibrin...) which are adapted to specific cell types. Consequently, to transfect cells on a variety of support, OZ Biosciences has developed specific reagents.



HOW DOES IT WORK?

Based on a new technology, the 3D transfection reagents allow to genetically modify cells directly cultured in 3D environment with high efficiency. 3D Transfection allows for a long term transgene expression (intracellular or secreted) or gene silencing. First, the nucleic acids (DNA, siRNA) are mixed with the 3D transfection reagent to form complexes. Then, those complexes are combined with the appropriate 3D matrices. Finally, the modified 3D matrices are colonized by cells to be transfected.



WHAT ARE THE APPLICATIONS AND STUDIES?

Tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth, angiogenesis, tube and acini formation...3D matrices are routinely used in basic research and therapeutic applications. The 3D transfection reagents allow genetic modification of cells directly into or onto the matrices and thus in a more natural environment.

3D-Fect™ and si3D-Fect™ for 3D-Scaffolds

The 3D-Fect™ transfection reagents were specifically designed to bind and cover any kind of 3D scaffold.

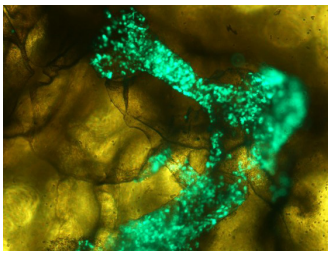
3D-FectIN™ and si3D-FectIN™ for Hydrogels

3D-FectIN™ transfection reagents are compatible with any hydrogel and allows transfecting cells directly cultured onto/into a hydrogel with a high efficiency. It does not alter gelation or polymerization.

1. Schmeichel KL, Bissell MJ. Modeling tissue-specific signaling and organ function in three dimensions. J Cell Sci 2003; 116: 2377–2388.
2. Scherer F et al. Nonviral vector loaded collagen sponges for sustained gene delivery in vitro and in vivo. J Gene Med 2002; 4: 634–643.

3D-Fect™ – A new outlook for your cells!

3D-Fect™ is a novel reagent, based on an innovative technology, specifically developed to directly transfect cells cultured in 3D scaffold.
3D matrices not only add a third dimension to cells environment, they also allow creating significant differences in cellular characteristics and behavior. In this way, scaffold-based 3D matrices combined with 3D-Fect/DNA complexes are colonized by cells to be transfected in a more natural environment.



APPLICATIONS

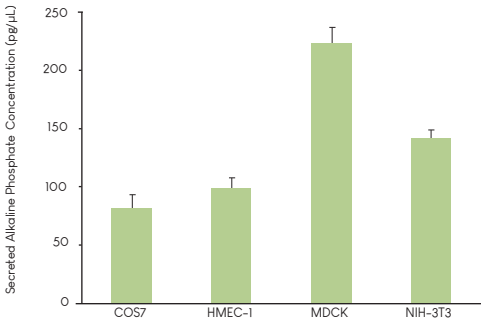
•**Perfect for all tranfection applications in 3D scaffolds such as sponges, matrices, inserts**: Tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth....

RECOMMENDED APPLICATIONS
DNA transfection of cells growing in 3D-scaffolds

MAIN FEATURES

- •**Highly efficient on** cell lines and primary cells
- •**Long term protein expression**:
3D-Fect™ allows 3D trangene expression studies in *in vivo* like conditions over a long time period
- •**Compatible with all types of nucleic acids**
- •**Gentle to cells:** 3D-Fect™ is biodegradable and allows high cell viability. It is serum compatible, thus no medium change is required

Alkaline phosphatase secretion of cells transfected into 3D Scaffold



Collagen-derived scaffolds were pre-loaded with complexes formed by 1 µg of pVectOZ-SEAP and 4 µL of 3D-Fect reagent. Cells were then seeded and secreted alkaline phosphatase (SEAP) concentration was measured after 48H.

Examples of 3D-Scaffolds successfully tested

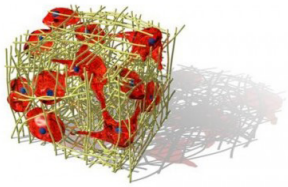
Collagen	Collagen-based Scaffold
Collagen-derived	Collagen-derived Scaffold
H.A	Hyaluronic Acid
Millicell™ (PTFE)	Cell Culture Insert (Millipore)
P.C.L	Polycaprolactone
P.E.G	Poly(Ethylene Glycol)
P.L.G.A	Poly(Lactic-co-glycolic acid)
P.S	Poly(Styrene)
P.U	Poly(Urethane)

Cat. No.	Product	Number of transfections with 1 µg of DNA
TF20250	3D-Fect 250µL	Up to 65
TF20500	3D-Fect 500 µL	Up to 125
TF21000	3D-Fect 1 mL	Up to 250

si3D-Fect™

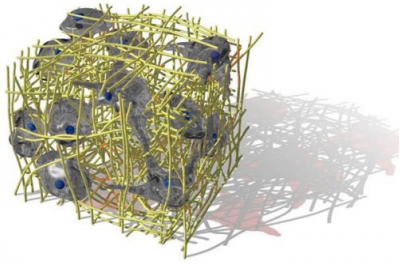
si3D-Fect™ is a 3D transfection reagent specifically designed and developed for silencing gene expression in cells cultured on 3D Scaffolds.

RECOMMENDED APPLICATIONS
Gene silencing of cells growing in 3D-scaffolds



MAIN FEATURES

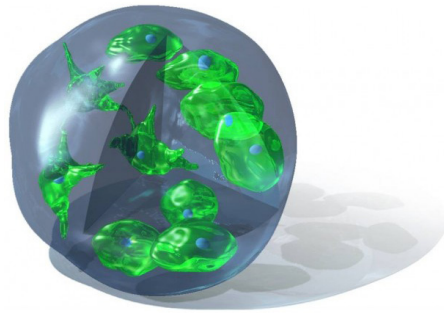
- •**Dedicated to short nucleic acid sequences** (siRNA, miRNA...)
- •**Long term gene silencing**
- •**Universal** (primary cells and cell lines)
- •**Serum Compatible**



Cat. No.	Product	Number of transfections with 50 nM siRNA in 100µL
STF40250	si3D-Fect 250µL	65 -125
STF40500	si3D-Fect 500 µL	125-250
STF41000	si3D-Fect 1 mL	250-500

3D-FectIN™ – A novel perspective for your cells!

3D-FectIN™ is the newest 3D-Transfection Reagent specifically developed to directly transfect cells cultured in 3D hydrogels. 3D-FectIN™ is suitable for all kind of hydrogels and cells.
3D matrices allow cells to grow in a micro-environment that more closely mimics the 3D environment encountered by cells *in vivo*. Thus, hydrogel-based 3D matrices combined with 3D-FectIN/DNA complexes allow cells to be directly transfected in more natural surroundings.



APPLICATIONS

- **Perfect for all transfection applications in 3D hydrogels:** Angiogenesis, tube and acini formation, colonization, neurite growth, tissue engineering & regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation...
- **Suitable for all type of nucleic acids including:** Plasmid DNA, linearized DNA, double stranded RNA, mRNA, oligonucleotides

MAIN FEATURES

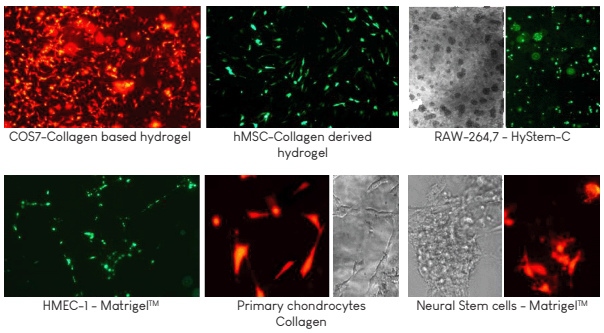
- **Highly efficient** on cell lines and primary cells
- **Compatible with all types of nucleic acids**
- **Long term protein expression**
- **Non-toxic and serum compatible**

Examples of 3D Hydrogels

Collagen	Collagen-based Hydrogels
Collagen-derived	Collagen-derived Hydrogels
H.A	Hyaluronic Acid
Gelatin	Extracellular Matrix (ECM)
Fibrin/ Fibronecin	ECM
Fibrinogen	ECM
Laminim	EECM
Matrigel™	BD Bioscience
Poly-(Ethylene glycol)	PEGylated hydrogels

RECOMMENDED APPLICATIONS
Transfection of cells growing in 3D-hydrogels

Transfection of various cells on different gels with 3D-FectIN

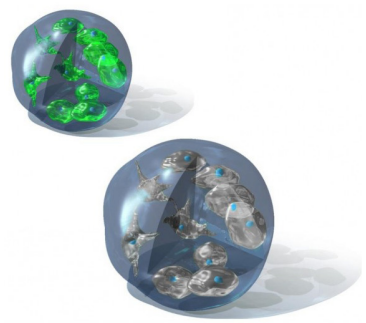


Cat. No.	Product	Number of transfections with 1 µg of DNA
TN30250	3D-FectIN 250 µL	Up to 65
TN30500	3D-FectIN 500 µL	Up to 125
TN31000	3D-FectIN 1 mL	Up to 250

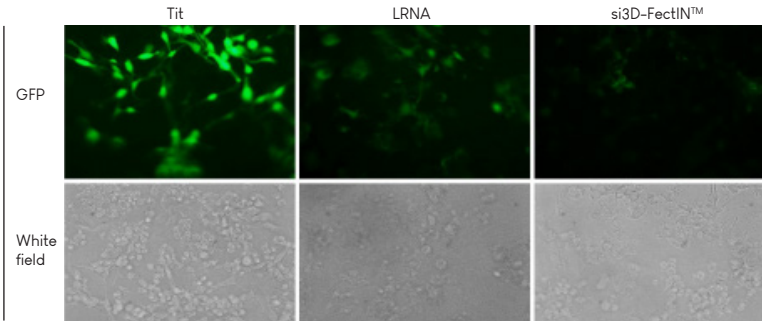
si3D-FectIN™

si3D-FectIN™ is a 3D transfection reagent specifically designed and developed for silencing gene expression in cells cultured in gels (or hydrogels).

RECOMMENDED APPLICATIONS
Transfection of cells growing in 3D-hydrogels



si3D-FectIN outperforms competitors



si3D-FectIN™ allows a more efficient gene silencing at very low concentrations of siRNA

Cat. No.	Product	Number of transfections with 50 nM siRNA in 50µL Gel
STN50250	si3D-FectIN 250 µL	30-65
STN50500	si3D-FectIN 500 µL	65-125
STN51000	si3D-FectIN 1 mL	125-250

CRISPR

Viro CRISPR

Prodel CRISPR

Poly CRISPR

RmesF CRISPR

Cas 9

PROTEIN DELIVERY

ProDeliverIN CRISPR / Rphy

AB-DeliverIN / FITC

SelfMag Kit

IN VIVO TRANSFECTION

In Vivo Magnetofection™

In Vivo Transfection

In Vivo DogtorMag

In Vivo CombiMag

In Vivo PolyMag

In Vivo Infection

In Vivo Viromag

In Vivo Gene Silencing

In Vivo SilenceMag

In Vivo Delivery into small animal brain

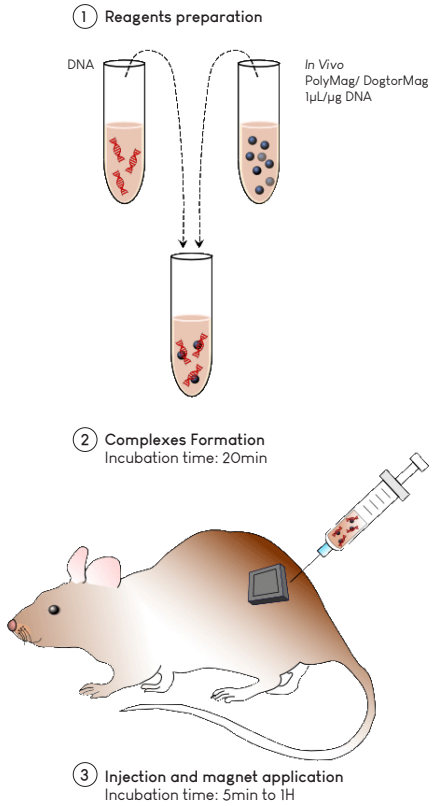
BrainFectIN™

In Vivo Magnetofection™ – The unique solution for *in vivo* targeted gene delivery

The main problems currently associated with systemic gene vector administration (gene therapy) include biodistribution of gene vector throughout the body, the lack of specificity towards a pathological site (bioavailability at the target site), the necessity of a large dose to achieve high local concentration, non-specific toxicity, inactivation of vectors due to undesired interactions with components of the *in vivo* milieu and other side effects due to high vector doses.
Magnetofection™ Technology resolves the problems related to diffusion limited process and to restricted bioavailability at the target site.

PRINCIPLE

In vivo Magnetofection™ has been designed for *in vivo* targeted transfection and infection.
This original system combines magnetic nanoparticles and nucleic acid vectors that will be retained after injection at the magnetically targeted site. In this way, targeted delivery minimizes systemic distribution and reduces toxicity.
Furthermore, the magnetic force will enhance the uptake of magnetic nanoparticles by the target tissue, and thus improve the efficiency of transfection/transduction. This allows reducing the required nucleic acid or virus doses and the process time of delivery which is crucial for improvement of *in vivo* nucleic acid delivery.



WHAT ARE THE APPLICATIONS?

Three optimized *in vivo* Magnetofection reagents have been designed according to defined applications:

Non viral applications

***In vivo* PolyMag -** a cationic polymer-based magnetic nanoparticles formulation - and ***in vivo* DogtorMag** - a cationic lipid-based magnetic nanoparticles formulation have been designed for *in vivo* targeted transfection of various types of nucleic acids such as DNA, RNA and oligonucleotides.
***In vivo* SilenceMag** is a rapid, simple and highly efficient method dedicated to transfect small RNA (siRNA, miRNA) into target cells/ tissue *in vivo*.

Viral applications

***In vivo* ViroMag** is an optimized nanoparticles formulation dedicated to viral vectors that allows reduction of titer virus. It is particularly suitable for Lentiviral/Retroviral, Adenoviral and Adeno-Associated Viral (AAV) vectors.

Target tissue	Route of injection	Site of injection	Kind of magnet	Magnet position
Tumor	Intravenous Intratumoral	Tail vein Tumor	All kind	**External** (subcutaneous tumor, brain tumor, well localized tumor) **Internal** (interne organ tumor)
Endothelial cells	Intra-arterial	Vessel of interest Ear artery Femoral artery	All kind	**Internal** (deep vessels) **External** (ear artery)
Heart	Intravenous Intra-arterial	Tail vein Carotid artery	Cylinder	**Internal** (in the chest) **External** (on the chest)
Liver	Intravenous Intra-arterial	Tail vein Carotid artery	Cylinder Square	**External** (on the right fiank) **Internal** (for focalized gene transfer)
Lung	Intravenous	Tail vein	Square	**External**
Intestine	Ileum lumen	Intestine	Cylinder, Square	**Internal**
Brain	Intraventricular	Brain ventricle	Small Cylinder	**External**

Magnet can be positioned:
- Externally for large organs or isolated organs (liver, brain, muscle, subcutaneous tumor)
- Internally for deep organs or focalized gene transfer

HOW DO I USE *IN VIVO* MAGNETOFECTION™ REAGENTS?

Gene vectors/nanoparticles complexes can be easily administrated through various injection routes such as:

- **Systemic administration** (intravenous, intra-artery)
- **Local administration** (intratumoral, intracerebroventricular, intraperitoneal, intramuscular, subcutaneous).

The only requirement for In Vivo Magnetofection™ is a small magnet specifically designed for this application. Several kinds of magnets are provided depending of your application.

In Vivo Transfection - *In Vivo* targeted gene delivery

In vivo Magnetofection™ has been designed for *in vivo* targeted transfection of various types of nucleic acids. This original system combines magnetic nanoparticles and nucleic acid vectors that will be retained after injection at the magnetically targeted site.
Two types of ready-to-use *in vivo* Magnetofection™ reagents are offered:

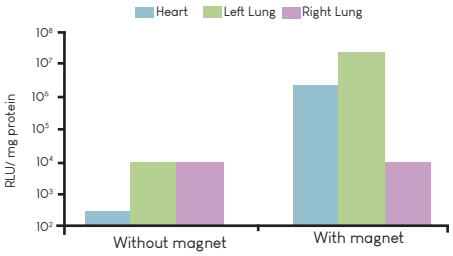
- ***In vivo* PolyMag** - a cationic polymer-based magnetic nanoparticles formulation.
- ***In vivo* DogtorMag** - a cationic lipid-based magnetic nanoparticles formulation. It associates *in-vivo* Dogtor, a specific cationic lipid and ***in vivo* CombiMag** magnetic nanoparticles.

APPLICATIONS

- **Suitable for various types of nucleic acids:** Plasmid DNA, siRNA, oligonucleotide, mRNA, shRNA, etc.
- **Several routes of administration:**
  - Systemic & Local administration

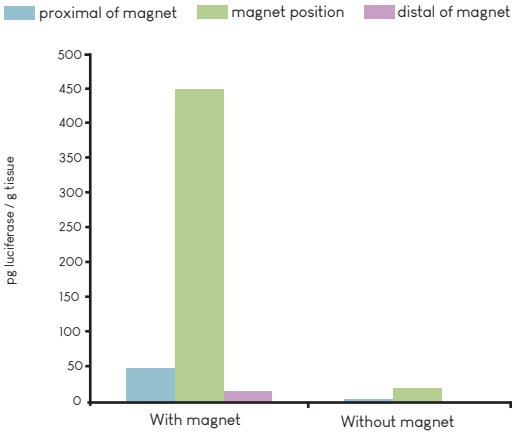
RECOMMENDED APPLICATIONS
In vivo targeted transfection

Transfection of heart and lung in mouse



Luciferase expression after tail vein injection, both in presence or absence of a magnet.

High efficiency of *In Vivo* PolyMag for targeted gene delivery after IV injection



After 42H, reporter gene expression was found primarily at the magnet position site and to a lesser extent proximal and distal of the magnet. As control, the same vector composition was injected in the contralateral vessel without application of a magnet. No significant reporter gene expression was found at the topographically analogous positions.
From Plank et al., Expert Opin Biol Ther., 2003; 3:745-58

MAIN FEATURES

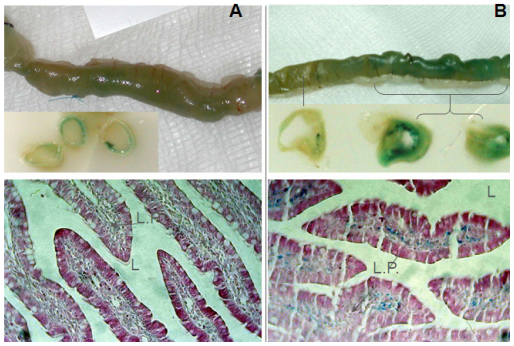
- **Increased transfection efficiency**
  The magnetic forces enhance the uptake of magnetic nanoparticles by the target tissue and thus improve the efficiency of transfection
- **Magnetically targeted transfection** to specific area
- **Reduction of the systemic dissemination** of vectors during injection. Targeted delivery minimizes systemic distribution, decreases gene vectors inactivation and reduces toxicity
- **Reduction of vector doses**
- **Work under non permissive conditions**
- **Universal - Suitable for all nucleic acids**
  Gene delivery/ODN delivery/Gene silencing
- **Non toxic**, biodegradable and totally biocompatible

PUBLICATIONS

"*Systemic delivery and activation of the TRAIL gene in lungs, with magnetic nanoparticles of chitosan controlled by an extrenal magnetic field*" **- *In Vivo* DogtorMag**
Ungureanu B.S. *et al* - **Int.J.Nanomedicine.2016**

"*Neuron-derived neurotrophic factor functions as a novel modulator that enhances endothelial cell function and revascularization processes*" **- *In Vivo* PolyMag**
siRNAs were injected into left adductor muscle of wild-type mice at the time of hindlimb ischemic surgery by using *in vivo* PolyMag magnetofection kit. During the procedure, magnet was placed on left adductor muscle for 20 min.
Ohashi K. *et al* - **J Biol Chem.2014**

Transfection with *in vivo* PolyMag in rat intestine



Complexes of DNA and in vivo PolyMag nanoparticles were injected into the ilea of rats in absence (A) or under the influence of a magnetic field (B)

Cat. No.	Product	Number of injections
IV-TK30210	In vivo PolyMag Trial Kit	1 cylinder Magnet + 100µL In vivo PolyMag
IV-TK30200	In vivo DogtorMag Trial Kit	1 Cylinder Magnet + 100µL In vivo Dogtor & in vivo CombiMag
IV-PN30500	In vivo PolyMag 500 µL	5-50
IV-PN31000	In vivo PolyMag 1 mL	10-100
IV-DM30500	In vivo DogtorMag 500 µL	5-50
IV-DM31000	In vivo DogtorMag 1 mL	10-100
IV-KC30210	In vivo PolyMag Starting Kit	1 Magnets set + 500µL In vivo PolyMag
IV-KC30220	In vivo DogtorMag Starting Kit	Magnets set + 500µL In vivo Dogtor & in vivo CombiMag

Magnet set contains 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm) magnets

Magnetofection Technology - This reagent needs to be used with a specific magnet

In Vivo ViroMag – *In Vivo* infection

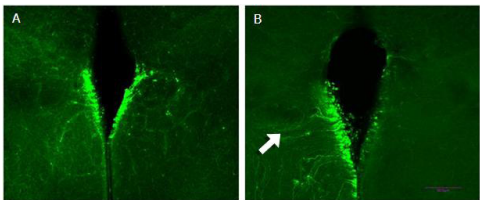
In vivo ViroMag has been designed to improve and target *in vivo* viral infection. This reagent is an optimized nanoparticles formulation dedicated to viral vectors.
This original system combines magnetic nanoparticles and viral vectors that will be confined at the magnetically targeted site after injection.

APPLICATIONS

- ***In vivo* transduction with all types of virus**
  Lentiviral/Retroviral, Adenoviral and Adeno-Associated Viral (AAV) vectors
- **Several routes of administration:**
  - Systemic & Local administration

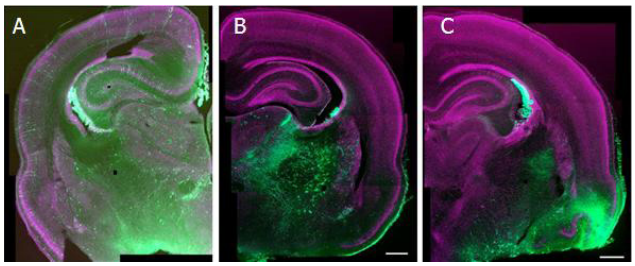
RECOMMENDED APPLICATIONS
In vivo targeted infection/transduction

High efficiency of *in vivo* ViroMag for targeting viral vector after intracerebroventricular injection.



Third ventricles of in utero rat embryos were injected with GFP-encoding viral particles alone or complexed to in vivo ViroMag. Without magnetic nanoparticles (A), the virus-transduced cells are located on both sides of the ventricle. Using *in vivo* ViroMag (B), transduction is enhanced and localized to one side due to a 30s magnet-application.

Infection of rat embryo Brain with Lentivirus



Infection of rat embryo brain. Brain sections at 8 days after lateral ventricular injection of 10^9 particles of GFP-lentivirus coupled with in vivo ViroMag into in utero rat embryos (E16) showed a diffuse GFP-expression (in green) due to a widespread infection of neurons (A). The association of GFP-lentivirus with ViroMag induced a targeted local area as shown by the GFP-expression in neurons lying under a magnet at the surface of the embryo skull (B). A more intense and restricted GFP-expression (C) was also observed when the magnet was positioned on the edge of the brain leading to an accumulation of viral particles and infected neurons in the focal area.

MAIN FEATURES

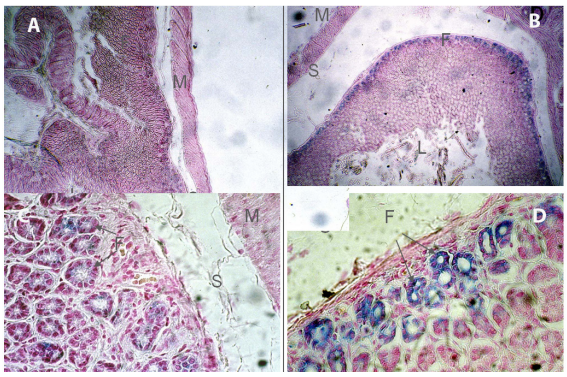
- **Increased transduction/infection efficiency**
  The magnetic forces enhance the uptake of magnetic nanoparticles by the target tissue and thus improve the efficiency of infection
- **Magnetically targeted transfection**
  to specific area
- **Reduction of virus titer & systemic dissemination**
  Targeted delivery minimizes systemic distribution, allows reduction of the vector doses and reduces toxicity
- **Work under non permissive conditions**
- **Non toxic**, biodegradable and totally biocompatible

PUBLICATIONS

Brain infection with Lentivirus
"*Virus stamping for targeted single-cell infection in vitor and in vivo*".
Schubert R. *et al* - **Nature Biotechnol.2018**

"*Magnetic nanoparticles for efficient cell transduction with Semliki Forest virus*".
Kurena B. *et al* - **J Viral Methods.2017**

High infection efficiency in mouse stomach with *in vivo* ViroMag



In the absence of a magnetic field, gene delivery occurred in only a few transfected cells (A,C), while exposure to a magnet for 20 min produces strong and widespread transgene expression (X-gal staining) in the crypts of the fundic glands 4 days after gene delivery (B,D)

Cat. No.	Product	Number of injections
IV-TK30230	In vivo ViroMag Trial Kit	1 Cylinder Magnet + 250µL in vivo ViroMag
IV-VM30250	In vivo ViroMag 250µL	10-25
IV-VM30500	In vivo ViroMag 500 µL	20-50
IV-KC30230	In vivo ViroMag Starting Kit	1 Magnets set + 250µL in vivo ViroMag

Magnet set contains 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm) magnets

Magnetofection Technology - This reagent needs to be used with a specific magnet

In Vivo SilenceMag – *In vivo* gene Silencing

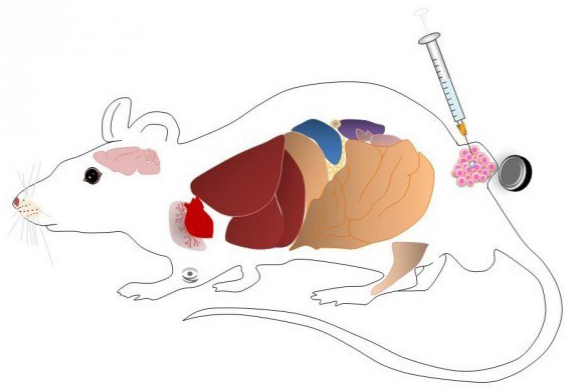
In vivo SilenceMag™ is a rapid, simple and highly efficient method dedicated to transfect small RNA (siRNA, miRNA) into target cells/tissue *in vivo*. It combines magnetic nanoparticles and small RNA that will be retained after injection at the magnetically targeted site. This targeted delivery method minimizes systemic distribution, increases gene targeted inactivation and reduces toxicity. Furthermore, the magnetic forces enhance the uptake of magnetic nanoparticles by the target tissue, and thus improve the efficiency of silencing.

This allows decreasing the required process time of delivery to few minutes which is crucial for improvement of *in vivo* small RNA delivery.
In vivo SilenceMag™ is designed to meet *in vivo* grade quality.

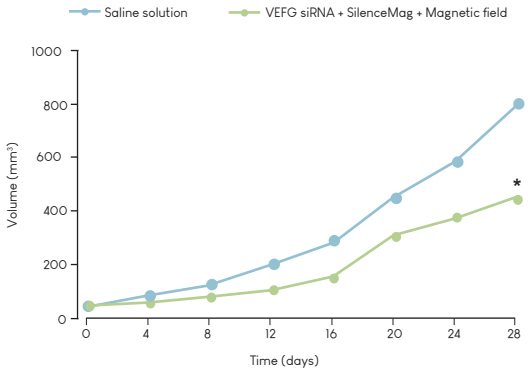
APPLICATIONS

- ***In vivo* gene silencing**
- **Several routes of administration:**
  - Systemic & Local administration

RECOMMENDED APPLICATIONS
In vivo gene silencing



Transfection of subcutaneous tumor in mouse



Subcutaneous tumors were generated by injection of hepatocarcinoma tumor cells into the right flank of immunosuppressed mice. Tumor growth was then monitored daily after intravenous injection of VEGF/SilenceMag
From Chen et al - BMC Cancer.2014

MAIN FEATURES

- **Increased silencing efficiency**
- **Targeted process** (magnetically-driven)
- **Reduction of the systemic** dissemination of siRNA/miRNA during injection
- **Reduction of the siRNA/miRNA doses**
- **Work under non permissive conditions** (hypothermia, physiological flow conditions)
- **Penetration of the siRNA/miRNA into tissues**
- **Minimized toxicity**

OZ Biosciences *in-vivo* magnets set:
1 extra small cylinder (ø 2 mm)
1 small cylinder (ø 5 mm)
1 cylinder (ø 10 mm)
1 square (18x18 mm)

PUBLICATIONS

"*Kidney-specific Csf2 knockdown. In vivo gene silencing achieved by transfecting siRNA using in vivo SilenceMag*".
Fujiu K. *et al* - **Nature Medicine.2017**

Cat. No.	Product	Number of injections
IV-TK30240	In vivo SilenceMag Trial Kit	Contains 1 Cylinder Magnet + 100µL reagent
IV-SM30500	In vivo SilenceMag 500 µL	5-50
IV-SM31000	In vivo SilenceMag 1 mL	10-100
IV-KC30240	In vivo SilenceMag Starting Kit	Magnets set + 500µL reagent

Magnet set contains 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm) magnets

Magnetofection Technology - This reagent needs to be used with a specific magnet

BrainFectIN™ – *In Vivo* delivery into small animal brain

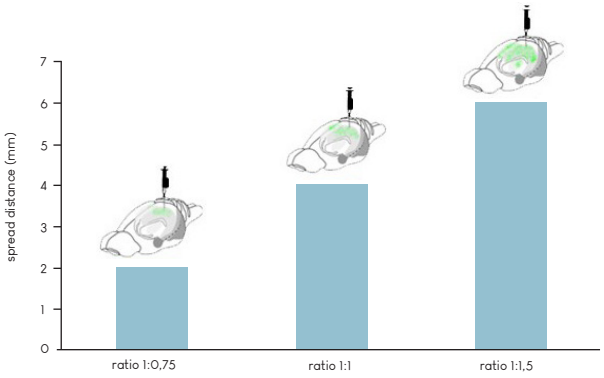
Major difficulties with gene delivery in the central nervous system is the weakness of standard non-viral gene carriers and the limitations associated to the use of viral particles (time-consuming and requires additional saftey precautions).
Unlike these methods, BrainFectIN™ is an original non-viral formulation that allows safe, easy and efficient nucleic acids delivery into central nervous system of small animals.

This transfection reagent allows transfection of neural cells in specific brain following stereotaxic injection, with low immunogenicity and rapid and long-term transgene expression.
BrainFectIN™ has been designed by our R&D team to meet *in vivo* grade quality (reagents performed under high manufacturing and quality standards and tested by strict quality controls).

RECOMMENDED APPLICATIONS

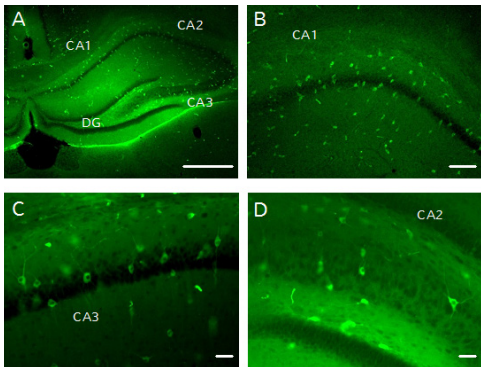
In vivo Nucleic Acids delivery-brain specific

Quantitative analysis of BrainFectIN™/DNA spread into the rat hippocampus



After injection, BrainFectIN™/pGFP complexes can spread into the whole hippocampus structure from rostro-caudal to lateral direction.

Stereotaxic injection of BrainFectIN/DNA complexes in hippocampus



GFP expression in hippocampus of rat 48h after BrainFectIN/pGFP injection (ratio 1:1.5). Scale bar = 100µm. The mix was injected through a nanofil needle implanted into hippocampus (stereotaxic injection). GFP+ cells are located in Dentate Gyrus (A) as well as hippocampal areas CA1 (A,B,C), CA2 (A) and CA3 (A,D). Negative control has been done with a stereotaxic injection of DNA alone in the same conditions. It shows a few cells transfected.

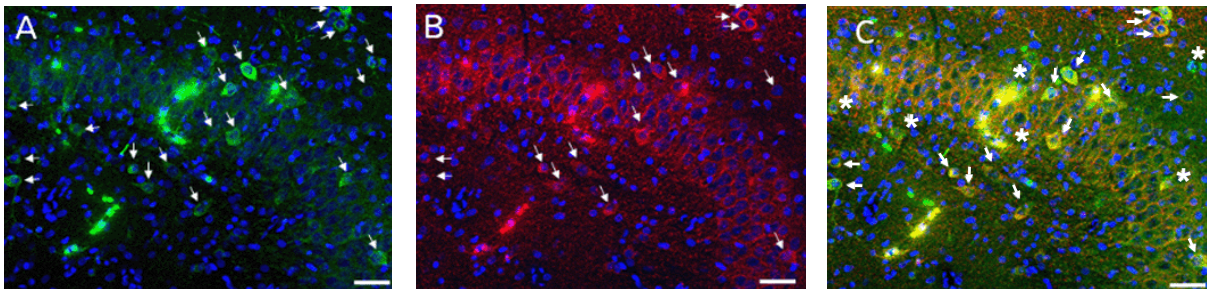
TESTIMONIAL

*"**BrainFectIN** was successfully tested in my lab for in vivo stereotaxic purpose and is now extensively used to modify neuronal cells.*
*This reagent **provides a new approach** leading to an efficient **transfection** rate and **a large diffusion scale** from ipsi to contralateral hemisphere by adjusting the injected volume. Our plasmid DNA- which can be detected for weeks after injection- is expressed shortly after transfection when **compared to a viral approach**.".*
Christophe P., PhD. - Neuroscience Center - INMED - University of Aix-Marseille - France

MAIN FEATURES

- **Targeted process** (stereotaxic injection)
- **Good transfection efficiency**
- **Reduction of the injection volume**
- **Reduction of the DNA doses**
- **Minimized toxicity**
- **Low immunogenicity**
- **Rapid and long-term transgene expression**

Double immunofluorescence staining performed in CA3 area



Transfected cells are GFP+ (A, arrows), and interneurons are labelled with GAD 65/67 (B, arrows), nuclei are counterstained with Hoecsht (A,B,C). Merge shows that we are able to transfect GABAergic interneurons (C, arrows). By exclusion, every other cell GFP+ is either pyramidal cell or hippocampal granule cell (C, asterix). It shows that BrainFectIN allows to transfect at least 3 different neural cell types after intra-hippocampal injection. Scale Bar = 50µm

Cat. No.	Product	Number of injections
IV-BF30100	In vivo BrainFectIN 100 µL	20-30
IV-BF30250	In vivo BrainFectIN 250µL	40-60
IV-BF30500	In vivo BrainFectIN 500 µL	80-120

VIRAL APPLICATION

Infection & Transduction Enhancers

Magnetofection Technology

- ViroMag
- ViroMag R/L
- AdenoMag

Chemical Formulations

- LentiBlast
- AdenoBlast

Cell sorting & Transduction

- ViroMICST

Capture, Concentration & Storage

- Mag 4C

Virus Production

- CaPo Transfection Kit

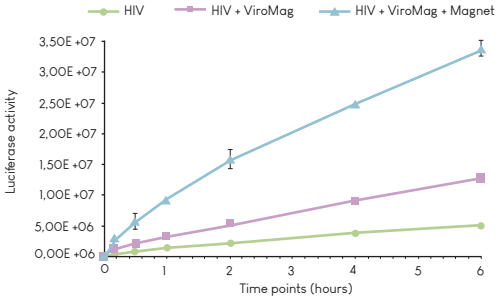
ViroMag – Viral transduction enhancer

ViroMag is a versatile reagent offering a solution for many viral applications. ViroMag and virus to be transduced are mixed in a one-step procedure; no molecular biology processes or biochemical modifications are required. This reagent demonstrates an exceptionally high efficiency to promote, control and assist viral transductions.
ViroMag is applicable to all viral vectors and presents unique properties due to a specific and optimized magnetic nanoparticles formulation.

APPLICATIONS

- **Suitable for all viral vectors**: Adenovirus, α-virus, Baculovirus, Herpes virus, Lentivirus, Retrovirus, Rhabdovirus, Paramyxovirus, Polyomavirus...
- **Mammalian cells**: Adherent and suspension primary cells, hard to transfect cells and cell lines

ViroMag accelerates HIV infection process on U87 – CD4 + cells

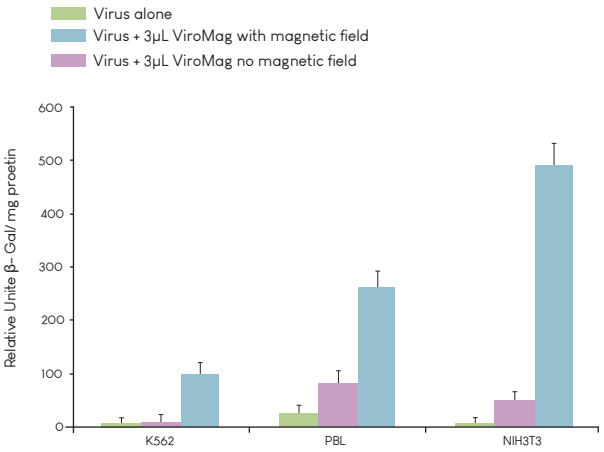


Successfully tested and published !

Browse our citation database online

RECOMMENDED APPLICATIONS
To increase viral transduction efficiency without Polybrene

ViroMag effect on Adenovirus transduction



ViroMag enhances adenoviral infection. K562, PBL and NIH-3T3 cells were infected with an adenovirus alone (Ad-LacZ) or with complexes of adenovirus and 3 µL of ViroMag. Cells were submitted or not to magnetic field and beta-Galactosidase expression was determined 24H after infection.

MAIN FEATURES

- **Increases viral transduction efficiency**
  Up to 500-fold gene expression enhancement compared to standard infection
- **Improves viral infectious capacity**
  Promotes infection even with very low viral titers/ doses
- **Concentrates viral dose, promotes and accelerates the infection process**
  Increases virus concentration from culture supernatant by 1000 to 4000 fold/Increases viral dose concentration on cell surface and uptake by 70-100 fold
  Restores transduction efficiency of PEGylated adenovirus by association
- **Extends the host tropisms to non permissive cells**
  Association of certain viruses with ViroMag is sufficient to force infection of cells lacking viral receptor
  Enhance ability to transduce in vitro target cells without modifying viruses
- **Allows synchronization of transduction**
  Synchronize viral cell adsorption (uptake)/ Accurately monitor the kinetics of viral replication cycle
- **Can provide a magnetic targeting**
  Magnetic-field guided local transduction: High transduction can be achieved under magnetic influence and confined to specific area by the magnet shape and position

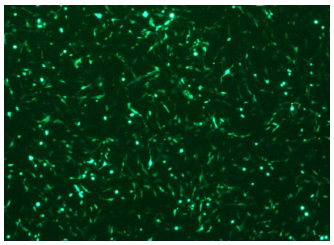
For in-vivo applications please refer to *in vivo* ViroMag page °

TESTIMONIAL

"Infection with ViroMag allowed to use 10 times less viruses with better infectivity and viability" .
Sloutskin A *et al* - **J Virol Methods. 2014**

"Synchronous infection of SIV and HIV in vitro for virology, immunology and vaccine-related studies" - **T Lymphocyte CD4⁺**
Sacha J.B. *et al* - **Nat Protoc.2010**

NIH3T3



Cat. No.	Product	Number of assays*
VM40100	ViroMag 100 µL	30-500 transductions in 96-well plate
VM40200	ViroMag 200 µL	60-1000 transductions in 96-well plate
VM41000	ViroMag 1 mL	300-50000 transductions in 96-well plate
KC30500	ViroMag Starting Kit	1 magnetic plate + 200 µL AdenoMag
KC30600	ViroMag Triple Starting Kit	1 magnetic plate + 100µL ViroMag, AdenoMag ViroMag RL

Based on MOI of 1 for 10^4 cells/well

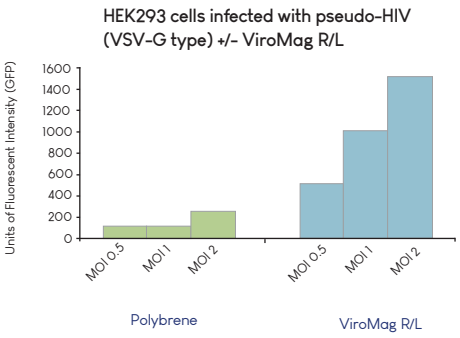
Magnetofection Technology - This reagent needs to be used with a magnetic plate

ViroMag R/L – Retrovirus & Lentivirus infection enhancer

ViroMag R/L Transduction Reagent is a magnetic nanoparticles formulation optimized for Retroviruses and Lentiviruses. Based on the Magnetofection™ technology, this reagent allows concentrating the complete applied dose of Retro/Lentiviral particles onto cells within minutes, inducing a significant improvement of virus infectivity with extremely low vector doses.

APPLICATIONS

- **Perfect for cell transduction with all retroviral and lentiviral vectors:** especially, VSG-G pseudo viruses
- **Suitable for mammalian cells:** Cell lines, primary cells, hard-to-transfect, suspension cells



Successfully tested and published !

Browse our citation database online

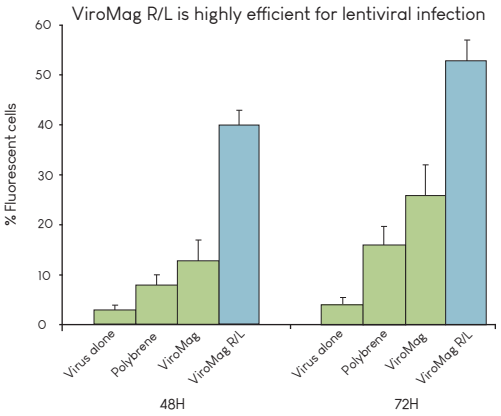
MAIN FEATURES

- **Increases viral transduction efficiency:** Up to 100-fold gene expression enhancement.
  Increases percentage of transduced cells
- **Improves viral infectious capacity**
  Significantly enhances virus infectivity even with very low viral doses/From 5 to 100-fold infectivity improvement
- **Concentrates viral dose**
  Increases retroviral titer from culture supernantant by 1000 to 4000 fold/Increases virus concentration on cell surface and uptake by 70-100 fold
- **Promotes and accelerates the infection process**
  Accelerates infection process
- **Allows synchronization of transduction**
  Synchronize viral cell adsorption (uptake)/Accurately monitor the kinetics of viral replication cycle
- **Straightforward and Non toxic**
  No molecular biology or biochemical processes required
- **Can provide a magnetic targeting**
  Magnetic-field guided local transduction: High transduction can be achieved under magnetic influence and confined to specific area by the magnet shape and position

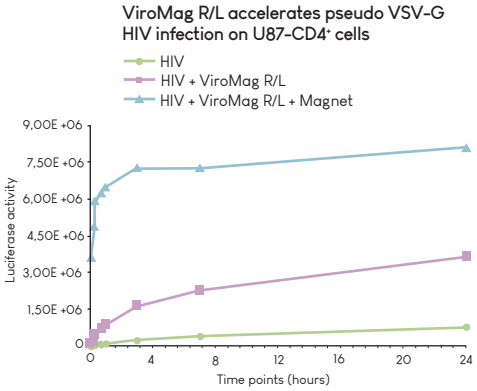
For in-vivo applications please refer to *in vivo* ViroMag page °

RECOMMENDED APPLICATIONS

Enhancing and synchronizing retro and lentiviruses transductions



ViroMag R/L is highly efficient for lentiviral infection. NIH-3T3 were infected with a lentivirus coding for GFP alone or with Polybrene, ViroMag and ViroMag R/L. Percentage of infected cells was determined 48 and 72H after infection by FACS analysis.



FOCUS ON

ViroMag R/L is ideal to infect non permissive cells

"High transduction efficiency on mammary epithelial organoids in suspension". Shamir E.R. *et al* - **J Cell Biol. 2014**

Cat. No.	Product	Number of assays
RL40100	ViroMag R/L 100 µL	30-500 transductions in 96-well plate
RL40200	ViroMag R/L 200 µL	60-1000 transductions in 96-well plate
RL41000	ViroMag R/L 1 mL	300-5000 transductions in 96-well plate
KC30700	ViroMag R/L Starting Kit	Contains 1 magnetic plate + 200 µL ViroMag R/L

Magnetofection Technology - This reagent needs to be used with a magnetic plate

AdenoMag - Adenovirus & AAV infection enhancer

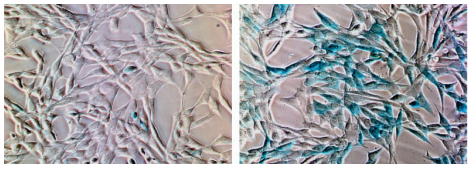
AdenoMag is a magnetic nanoparticles based reagent dedicated to enhance Adenovirus and Adeno Associated Virus (AAV) infection. It allows to concentrate rapidly all viral particles onto cells. AdenoMag permits to improve significantly virus infectivity with extremely low vector doses. Due to its specific properties, AdenoMag is ideal to infect non permissive cells. No molecular biology processes or biochemical modifications are required.

APPLICATIONS

- **Ideal for boosting your cell transduction with all adenoviral and AAV vectors**
- **Suitable for all mammalian cells:** Cells lines, primary cells, hard-to-transfect & non-permissive cells

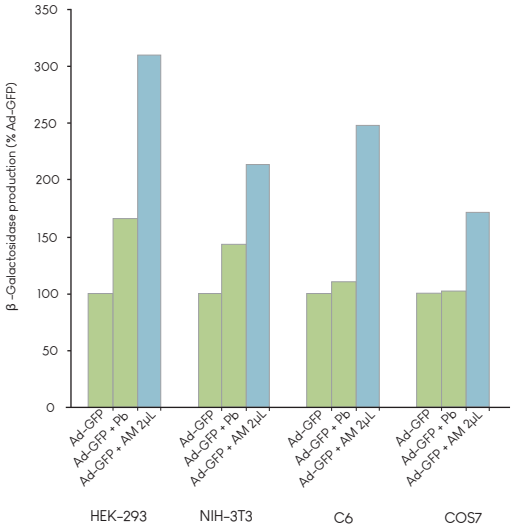
RECOMMENDED APPLICATIONS
Adenovirus and adeno-associated-virus transductions in vitro

Comparison of NIH-3T3 infection with or without AdenoMag



Virus alone | Virus + AdenoMag

Adenomag enhances transgene expression



beta-Galactosidase expression was determined in HEK-293, NIH-3T3, C6 and COS7 cell lines after 24H of infection with Ad-LacZ. Ad-LacZ with Polybrene (Ad-LacZ + Pb) or Ad-LacZ with 2 µL of AdenoMag (Ad-LacZ + AM).

MAIN FEATURES

- **Increases transduction efficiency:** the combination of superparamagnetic nanoparticles with adenovirus has shown up to 500-fold enhancement of gene expression compared with standard infection
- **Concentrates viral dose,** promotes and accelerates the infection process. Improves viral infectious capacity
- **Significant enhancement** of adenovirus infectivity can be achieved with the use of magnetic nanoparticles
- **Extends the host tropisms** of viral vectors to non-permissive cells. The association of viral vectors with magnetic nanoparticles is sufficient to permit infection of non-permissive cells
- **Provides a magnetic targeting**: high transduction efficiency can be achieved under magnetic influence and a specific targeting to define area can be done. Indeed, magnetic targeting localized to specific area linked to the magnet size and shape has been demonstrated for adenovirus and AAV

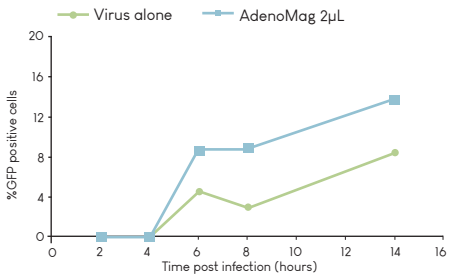
For in-vivo applications please refer to *in vivo* ViroMag page °

FOCUS ON

"Magnetofection™ is a successful method in enhancing viral transduction efficiency.

ViroMag, ViroMag R/L and AdenoMag, the 3 magnetofection reagents dedicated to viruses, enable to increase transduction efficiency up to 10 fold compared to virus alone.
In addition, this technology accelerates and synchronizes the transduction process, and enables to concentrate the viral dose onto cells for optmail performance".

NIH-3T3 cells infection kinetics (MOI = 1) +/- AdenoMag



Cat. No.	Product	Number of assays*
AM70100	AdenoMag 100 µL	500-1000 transductions in 96-well plate
AM70200	AdenoMag 200 µL	1000-2000 transductions in 96-well plate
AM71000	AdenoMag 1 mL	5000-10000 transductions in 96-well plate
KC30900	AdenoMag Starting Kit	Contains 1 magnetic plate + 200 µL AdenoMag

**Based on MOI of 1 for 10^4 cells/well*

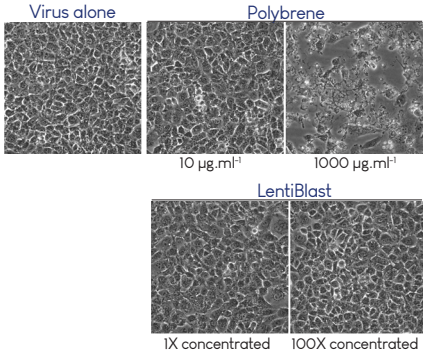
Magnetofection Technology - This reagent needs to be used with a magnetic plate

LentiBlast

LentiBlast™ Kit is ideal to enhance lentiviral infection and transduction in any type of cells, adherent or in suspension, primary or cell lines. Its patent-protected chemical composition allows simultaneously neutralizing electrostatic repulsions between membrane and viral particles and enhancing viral fusion with cell membrane.
Due to a favorable "membrane permeable effect" limiting the transmembrane potential changes, LentiBlast™ is totally compatible with cell viability. LentiBlast overcomes obstacles that prevent successful transduction (cell density, passage number, lentivirus purity, MOI ...).

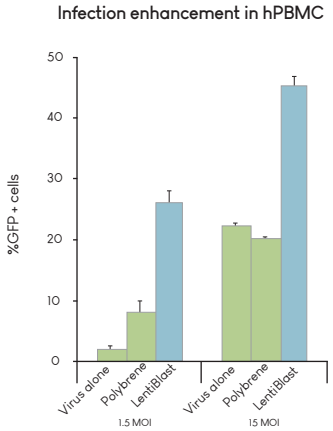
MAIN FEATURES

- **Enhances** infection and transduction efficiency of lentivirus
- **Compatible with cell lines and primary cells**
- **Allows** using reduced amounts of Lentivirus (low MOI)
- **Composed of two reagents for** a higher compatilbility and efficiency
- **Non-toxic** even at high concentration



RECOMMENDED APPLICATIONS

Enhancing lentiviral transduction in any type of cells



TESTIMONIAL

"We use LentiBlast to help achieve high transduction efficiency of human primary T cells. It has lower toxicity than polybrene which is traditionally use to enhance transduction and efficiency was doubled compared to Polybrene" .
Nina F - **Albert Einstein College of Medicine**

Cat. No.	Product	transduction in a 24-well plate
LB00500	LentiBlast 2x500 µL	Up to 100
LB01500	LentiBlast 2x1500 µL	Up to 300

AdenoBlast

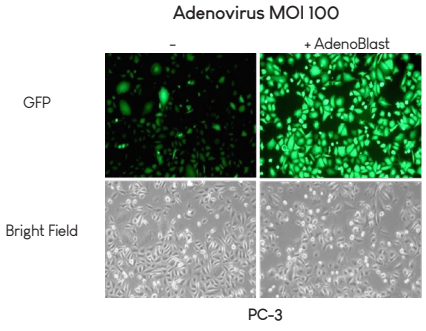
AdenoBlast™ is ideal to enhance adenoviral infection and transduction in any type of cells, adherent or in suspension, primary or cell lines. The use of adenoviral transduction requires the Coxsackie Adenovirus Receptor (CAR) for its initiation. Many cells express only low amounts of CAR making limited adenovirus-mediated transduction. AdenoBlast™ is based on an adenovirus binding peptide that assists transduction by coupling the adenoviral particles to the cell membrane in a CAR independent manner. Therefore adenoviral transduction is now possible even in non-permissive cells. AdenoBlast is especially recommended when high multiplicities of infection (MOI) are used.

MAIN FEATURES

- **Enhances** infection and transduction efficiency of adenovirus
- **Ideal for permissive and non-permissive cells**
- **Non-toxic** (potential for in vivo applications)
- **Compatible with cell lines and primary cells**
- **Allows** using reduced amounts of Adenovirus (low MOI), reducing the cost

RECOMMENDED APPLICATIONS

Increasing adenoviruses' transduction efficiency in any type of cells



Cat. No.	Product	transduction at 1x10⁵ IU
AB00125	AdenoBlast 125 µL	Up to 50
AB03125	AdenoBlast 3x125 µL	Up to 150

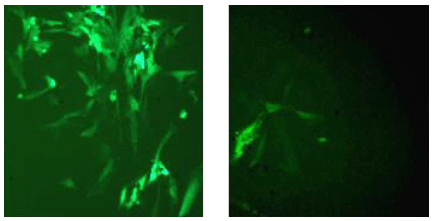
Viro-MICST™ - Efficient and specific target cells transduction

i-MICST™ Technology (integrated Magnetic Immuno-Cell Sorting and Transfection/Transduction) is a new platform that allows to genetically modify cells directly on magnetic cell purification columns. This technology combines cell isolation and genetic modification in one simple, efficient and reliable integrated system.
Designed for i-MICST™ Technology, the Viro-MICST™ reagent allows efficient and specific transduction of target cells directly on magnetic cell-purification columns (*for more information on i-MICST™ Technology see p°*).
Ideal for sensitive cell types such as primary and stem cells, Viro-MICST™ leads to an increase in the transduction efficiency with low-titer virus preparations compared to regular transduction methods.

APPLICATIONS

- **Suitable for all viruses:** including AAV, Adenovirus, Lentivirus and retrovirus
- **Ideal for mammalian cells:** Adherent and suspension cells, primary and hard-to-transfect cells, cells lines, sensitive cells

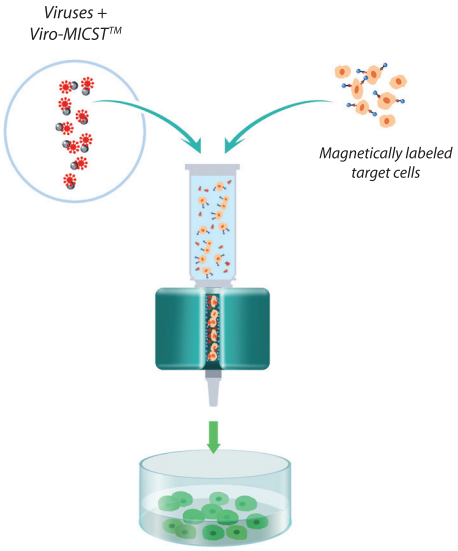
hUC-MSC adenoviral transduction improved by ViroMICST



Viro-MICST™ | Standard infection

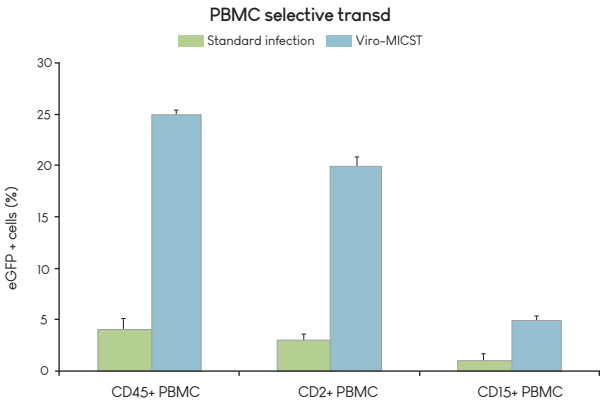
RECOMMENDED APPLICATIONS
Transduction/ Infection of cells during magnetic cell purification

Integrated Magnetic Immuno-Cell Sorting and Transduction in one single system



MAIN FEATURES

- **Isolation and transduction of cells in one reliable integrated system**
  Reduce cell manipulation steps, minimize cell stress and save time
  Ideal for sensitive cell types such as primary and stem cells
- **High and increased transduction efficiency**
  Benefit from high transduction efficiency with low Multiplicity of Infection (MOI) during magnetic cell separation
  Save vector material
- **Acceleration of the transduction process** and synchronization of adsorption
- **Cell phenotype maintained**
  Cells maintain their differentiation potential after using Viro-MICST procedure



Transduction with Viro-MICST. Human PBMC were labeled with either CD45, CD2 or CD15 microbeads. Each condition was then loaded into i) one unmodified MACS® LS column, and selected cells were then infected using standard lentiviral (standard infection) or ii) one unmodified MACS® LS column followed by a MACS® LS column modified with Viro-MICST/LV.eGFP complexes. Infection efficiency was measured by flow cytometry

FOCUS ON

"Magselectofection: an integrated method of nanomagnetic separation and genetic modification of target cells."
Sanchez-Antequera Y *et al* - **Blood.2011**

Cat. No.	Product	Number of transductions per small column*
VMX250	Viro-MICST 250µL	25-50
VMX500	Viro-MICST 500 µL	50-100
VMX1000	Viro-MICST 1000 µL	100-200

**Based on MOI of 1 for 10^6 labeled-cells/column*

Mag 4C – Magnetic Virus Concentration

Mag4C Kit is specifically designed and developed for capturing, concentrating and storing viruses. This kit is composed of 3 reagents allowing Magnetic Capture/Concentration, Elution and Conservation of viruses.

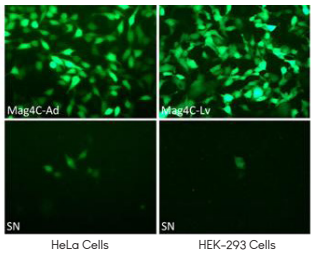
Mag4C magnetic nanoparticles capture viruses in culture media with 80-99% efficiency.
Once captured onto magnetic beads, viruses can be:
• Concentrated and stored with the Conservation Buffer or directly used for downstream assays
• Concentrated, eluted from the magnetic beads with the Elution Buffer and stored with the Storage Buffer or used for various assays

APPLICATIONS

- **Suitable for all conditions & viruses:** 2 different products available:
  - Mag4C-Ad for adenoviruses
  - Mag4C-Lv for lenti- & retro-viruses

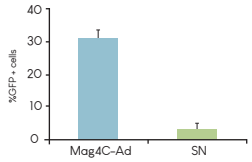
RECOMMENDED APPLICATIONS
Fast capture, concentration & conservation of viruses

Efficiently captures viruses

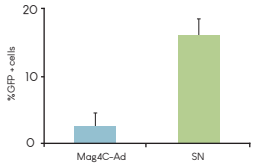


Mag4C beads efficiently captured virus since supernatants (SN) are nearly no more infectious (absence of virus) whereas viral particules bound to the Mag4C beads are highly infectious.

HEK-293 cells infection BEFORE Elution

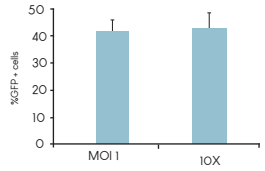


HEK-293 cells infection AFTER Elution



Elution is straightforward, easy and very efficient. Mag4C beads were no more infectious after the elution procedure.

%HeLa infection after Concentration



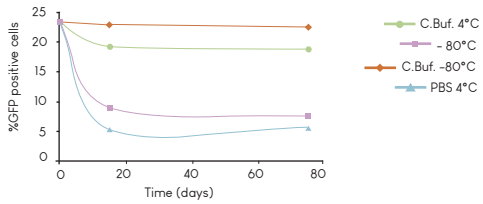
Captured, eluted and concentrated virus is as infectious as untreated virus.

MAIN FEATURES

- **Concentration** viruses by magnetic capture in 30-45 minutes
- **Obtain high yield** of viral capture and recovery
- **Reduce handling steps :** Avoid ultracentrifugation precipitation and chemicals
- **Mag4C** beads improve transduction efficiency (Magnetofection advantages)

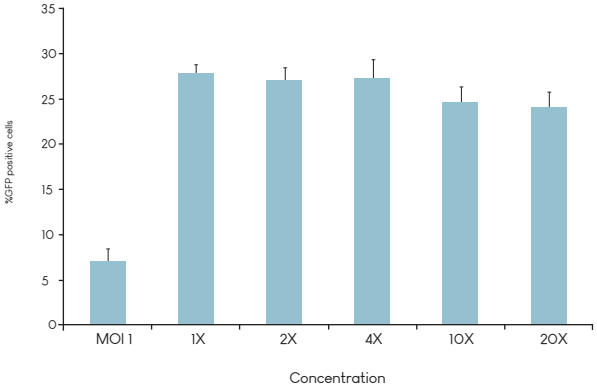
Learn More: Mag4C Technology description page °

% COS7 infection after Conservation in Conservation Buffer or PBS



Viral particles stored in conservation buffer maintain high infectivity over long-term storage.

% of COS7 infected after capture and concentration



After capture, virus complexed to Mag4C beads can be concentrated without losing transduction efficiency

CONSERVATION BUFFER

Mag4C Conservation Buffers (Lv&Ad) have been expressly designed to improve the stability of viruses upon storage conditions and are fully compatible with the magnetic nanoparticles.

Mag4C-Ad Kit - specific for Adenovirus
Cat # ATK11200 - Mag4C-Ad beads (0.2 ml) + Elution buffer (5mL) + Conservation buffer (0.2 mL) - Up to 20 assays
Cat # AKC11000 - Mag4C-Ad beads (1 mL) + Elution buffer (5mL) + Conservation buffer (1 mL) - Up to 100 assays

Mag4C-LV Kit - specific for Lentivirus & Retrovirus
Cat # LTK11200 - Mag4C-Lv beads (0.2 ml) + Elution buffer (5mL) + Conservation buffer (0.2 mL) - Up to 20 assays
Cat # LKC11000 - Mag4C-Ad beads (1 ml) + Elution buffer (5mL) + Conservation buffer (1 mL) - Up to 100 assays

A Multipurpose Magnetic Separation Rack for 50, 15 or 1.5 mL tubes is also proposed. It can hold 12 standard microtubes, two 15 mL and two 50 mL tubes.

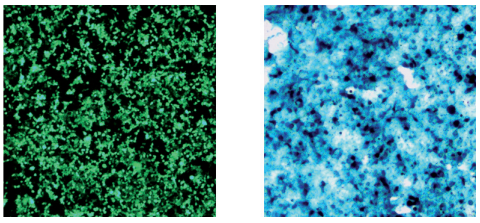
Calcium Phosphate Transfection Kit - Virus Production

Calcium Phosphate Transfection Kit is perfect to transfect HEK 293 cells. This transfection method, first described by Graham and Van Der Ebb in 1973, has been optimized in order to reach higher transfection efficiency.
The CaPO transfection kit is simple and easy to use. It allows reaching between 95 and 100% of HEK 293 transfected cells and a very high titer for virus production.

APPLICATIONS

•**The ideal method for HEK 293 cells transfection:** Calcium Phosphate Transfection Kit is optimized for the transfection of HEK 293 cells with plasmid DNA CaPO Transfection Kit is also appropriate for a variety of immortalized cell lines such as CHO and COS

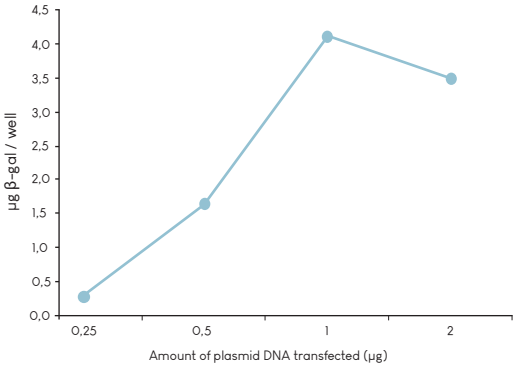
HEK-293 cells trtansfected with the calcium Phosphate Transfection Kit



GFP β-galactosidase

RECOMMENDED APPLICATIONS
Transfection of HEK293 cells for production of viral vectors and proteins

Protein production with the Calcium Phosphate Transfection Kit



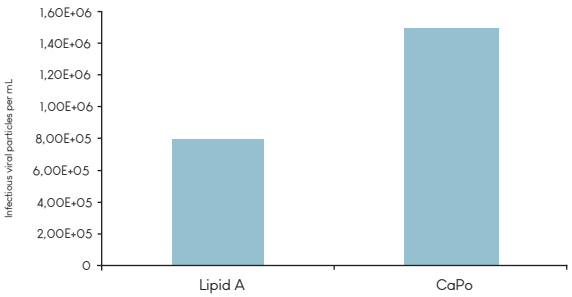
HEK 293 cells were prepared and transfected in 24-well plates with several amount of a pLACZ plasmid encoding β-Galactosidase. The amount of β-galactosidase produced per well was determined by ONPG assay.

MAIN FEATURES

- **Ideal for virus production**
- **High HEK 293 cells transfection efficiency**
- **Suitable for producing recombinant proteins**
- **Serum compatible**
- **Simple, Ready-to-use and Rapid:**

1. Plate the cells in DMEM and incubate overnight
2. Change tissue culture medium 1-2H before transfection
3. Prepare the DNA solution in 1X HBS
4. Add the Calcium Chloride solution, mix and incubate 30 min
5. Add the complexes drop wise to your cells

Virus production with CaPO kit versus competitor



A Lentiviral expression plasmid, a packaging plasmid, and a pseudotyping plasmid were mixed together (20 µg total DNA amount) and transfected in a 100 mm dish with the CaPO kit. As a control the same amount of the three plasmids were transfected with a competitor's reagent as indicated by the manufacturer's instruction manual. Viral particles were collected after 48H and viral titers were determined using HeLa-CD4 β-galactosidase cells (MAGI assay).

FOCUS ON

Principal Calcium Phosphate Transfection Kit advantages:

•Compaction of DNA in nanoparticles efficiently internalized by cells

•Protection of nucleic acids against nucleases degradation

•Modified and optimized to reach higher transfection levels

Cat. No.	Product	Number of assays
CP90000	CaPO Transfection Kit	100 in 100mm culture dishes with 1µg of DNA

Kit content: 1X Hepes Buffered Saline 4x15 mL+2.5 M CaCl2 3.5 mL

CELLULAR ASSAY KITS

Protein Dosage

FluoProdige Assay Kit / Bradford Protein Assay

Reporter

CPRG / ONPG / X-Gal / SEAP / Luciferase

Viability

MTT / OZBlue / ROS / Senescence

TRANSFECTION TOOLS

Luciferin

G418 / X-Gal

GeneBlaster / Blast / MTX Boost / pVectoz

Vaccine Adjuvants

TECHNICAL APPENDIX

List of cells successfully transfected

Transfection troubleshooting

Protocols: How to optimize ?

Need-to-know information