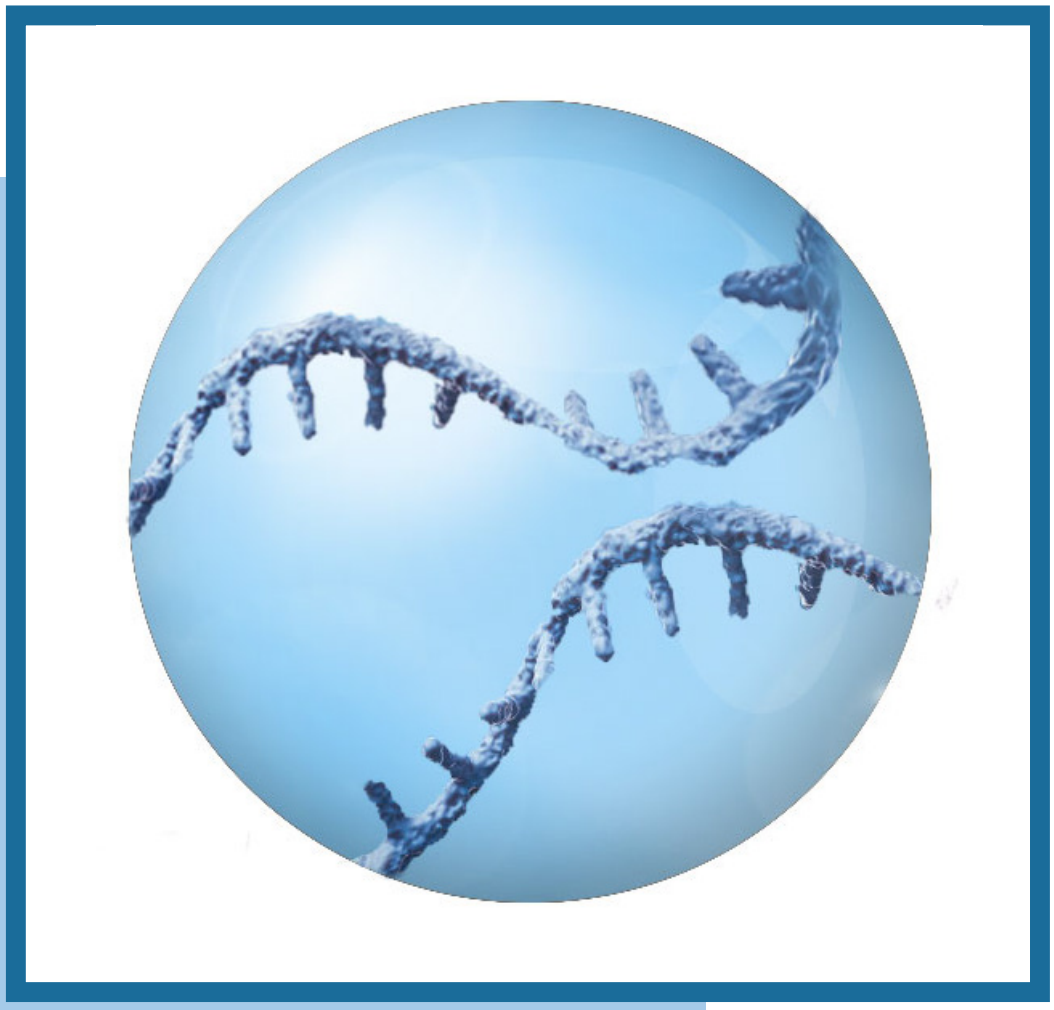


mRNA

HIGH QUALITY PRODUCTS
&
CUSTOM SERVICE

Why choose mRNAs over pDNA?

Vaccine, Gene Reporter,

Genome Editing & Gene Replacement mRNAs

OZ BIOSCIENCES
The art of delivery systems

afaq ISO 9001 Qualité AFNOR CERTIFICATION

Transfect With Stabilized mRNA Instead Of Plasmid DNA

Why choose stabilized mRNA over pDNA?

ADVANTAGE #1: It does not require nuclear uptake for being expressed since translation of mRNA occurs into the cytoplasm. Indeed, nuclear delivery (passing through the nuclear membrane) is one of the principal barriers for transfecting slow or non-dividing cells and consequently, mRNA transfection is particularly attractive for such purpose.

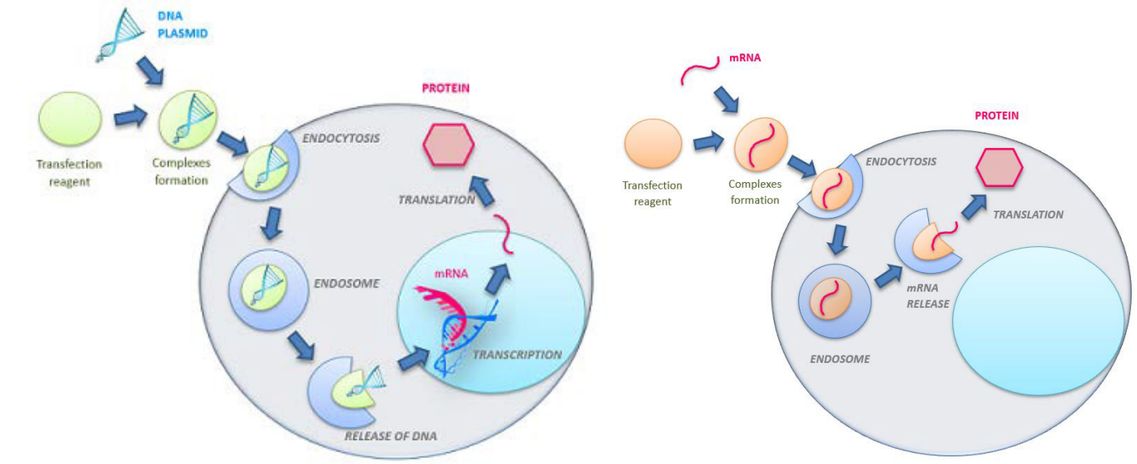


Figure 1: Protein translation process following DNA and mRNA transfection

ADVANTAGE #2: This approach is not integrative. Contrary to pDNA, mRNA cannot lead to genetic alteration.

ADVANTAGE #3: Perfect for hard-to-transfect cells. mRNA has several merits over DNA that allows to genetically modify primary and hard-to-transfect cells more easily. Beside the fact that with mRNA there is no risk of integration into the host genome, mRNA transfection is cell cycle-independent, particularly suitable for slow-dividing cells such as endothelial cells or dendritic cells[1].

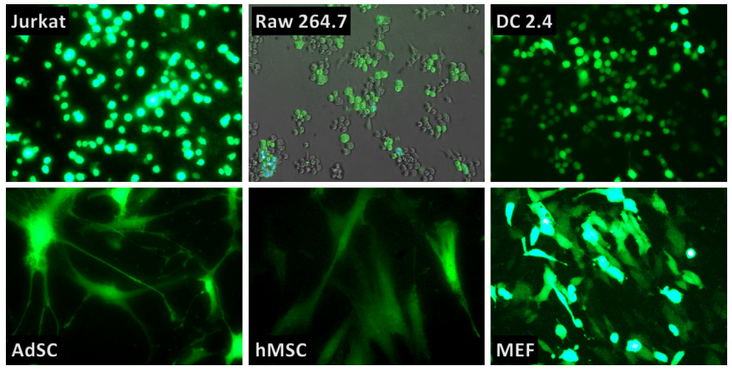


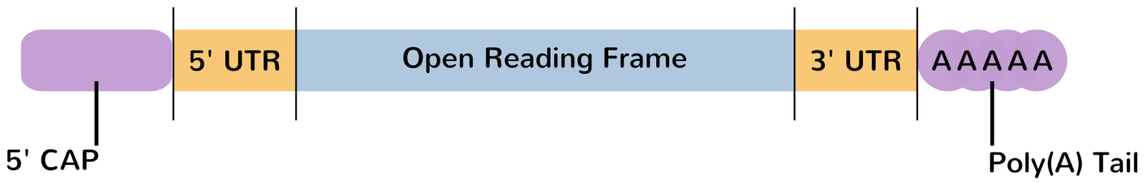
Figure 2: Jurkat T cells, Raw264.7, DC2.4, primary human Adipose Stem Cells, primary human Mesenchymal Stem Cells and Mouse Embryonic Fibroblasts were transfected using RmesFect transfection reagent and mRNA-GFP from OZ Biosciences.

1 Yamamoto A. et al, Eur J Pharm Biopharm. 2009, Current prospects for mRNA gene delivery

mRNA Complete Benefits

- No need for nuclear uptake - protein expression directly in cytoplasm
- Faster protein expression than DNA transfection
- No genomic integration
- Perfect for transfecting slowing or non-dividing cells
- Protein expression in a total promoter-independent manner
- Transient transfection: mRNA based expression of proteins sustains for a limited time

mRNA Structure



5′ Cap

This cap structure protects mRNA from degradation and recruits processing and translation factors. In mammals, the predominant form is a 7-methyl-guanosine (Cap 0) linked via an a 5′ to 5′ triphosphate bridge to the first transcribed nucleotide which is methylated on the ribose O-2 position (Cap 1).

5′ Untranslated Region (5′ UTR)

The 5′ UTR is a non-coding region directly upstream from the initiation codon involved in the post- transcriptional regulation of gene expression by modulating mRNA stability, transport, subcellular localization and translation efficiency thus allowing a fine control of the protein product. This region has a high GC content and several secondary structures and comprises the Kozak sequence (GCCGCCRCCAUGG) that plays a major role in the initiation of the translation process.

Open Reading Frame (ORF)

This internal region of eukaryotic mRNA is translated into protein. The ORF begins with a methionine codon (AUG) and ends with a stop codon.

3′ Untranslated Region (3′UTR)

The 3′ UTR is the part of mRNA that immediately follows the translation termination codon. This region plays a crucial role in gene expression by influencing the localization, stability, export, and translation efficiency of an mRNA.

Poly(A) tail

The poly(A) tail is a long sequence of adenine nucleotides (0-250 nucleotides with a median length of 50- 100 in HeLa and NIH-3T3 cells)[2] added to the 3′ end of the pre-mRNA.
The poly(A) tail contains binding sites for poly(A) binding proteins (PABPs) that play a major role in export from the nucleus, translation, and protection from degradation. Its length is an important determinant of translational efficiency and mRNA stability. This is an important element as its absence or removal often leads to exonuclease-mediated degradation of the mRNA.

2 Chang H. et al; Mol Cell 2014 - PMID: 24582499A

Vaccine mRNAs

Nucleic acid Vaccine combine the positive features of live attenuated vaccines while avoiding many potential safety limitations[3]. These vaccines present several advantages over conventional vaccines such as:

- Mimicking a live infection by expressing antigens in situ after immunization and priming both B and T cell responses including cytotoxic T lymphocytes[4].
- Revealing focused immune responses directed toward the selected antigens of interest with no potential reversion to pathogenicity.
- Serving the dual purpose of expressing the desired antigen as well as acting as an adjuvant.
- Having a superior safety profile compared to inactivated viruses or pathogens.

mRNA Vaccines Benefits

mRNA vaccines present a better safety profile than DNA vaccines: DNA vaccines display a long term expression, a potential risk for genome integration and induction of anti-DNA antibodies[5]. The main advantages of mRNA vaccines come from the intrinsic properties of the mRNA:

- They are produced using cell-free enzymatic transcription.
- The transient expression of mRNA encoded antigen enables a more controlled antigen espression and minimizes the risk of tolerance induction that can be associated with long-term exposure.[6]
- There is thus an absence of any additional encoded protein which exclude the possibility of raising undesired immune response or interaction with the host.[7]
- Their stabilized design allows higher level of expression in vivo.[8]

OZ Biosciences mRNAs for mRNA Vaccine:

OVA mRNA - ref# MRNA41 (5moU) - ref# MRNA42 (Unmodified) - ref# MRNA40 (N1-mψ)	Designed to produce high expression level of Ovalbumin Protein. That is a commonly used antigen for immunization and biochemical studies.
Spike SARS-CoV-2 mRNA - ref #MRNA35 (5moU) - ref #MRNA34 (Unmodified) - ref# MRNA43 (N1-mψ)	Designed to produce high expression level of Spike Protein of SARS-COV-2 virus. That is a commonly used antigen for immunization and biochemical studies.
Spike DELTA mRNA - ref #MRNA37 (5moU) - ref #MRNA36 (Unmodified) - ref# MRNA45 (N1-mψ)	Designed to produce high expression level of DELTA Mutant Spike Protein of SARS-CoV-2 virus.
Spike OMICRON mRNA - ref #MRNA39 (5moU) - ref #MRNA38 (Unmodified) - ref# MRNA44 (N1-mψ)	Designed to produce high expression level of OMICRON Mutant Spike Protein of SARS-CoV-2 virus.
N SARS-Cov-2 mRNA - ref #MRNA53 (5moU) - ref #MRNA52 (Unmodified) - ref# MRNA54 (N1-mψ)	Designed to produce high expression level of the nucleocapsid protein of SARS-CoV-2 virus.
HA-H1N1 mRNA - ref #MRNA47 (5moU) - ref #MRNA46 (Unmodified) - ref# MRNA48 (N1-mψ)	Designed to produce high expression level of the (HA) hemagglutinin surface glycoproteins of H1N1 subtype of Influenza A virus.
HA-H3N2 mRNA - ref #MRNA50 (5moU) - ref #MRNA49 (Unmodified) - ref# MRNA51 (N1-mψ)	Designed to produce high expression level of the (HA) hemagglutinin surface glycoproteins of H3N2 subtype of Influenza A virus.

3 Deering RP et al, Expert Opin Drug Deliv. 2014. Nucleic acid vaccines: prospects for non-viral delivery of mRNA vaccines
4 Johansson DX et al, PLoS One. 2012. Intradermal electroporation of naked replicon RNA elicits strong immune responses.
5 Pascolo S., Handb Exp Pharmacol. 2008.Vaccination with messenger RNA (mRNA).
6 Pollard C. et al, Trends Mol Med. 2013. Challenges and advances towards the rational design of mRNA vaccines.
7 Schlake T. et al, RNA Biol. 2012. Developing mRNA-vaccinetechnologies.
8 Kallen KJ. et al, Hum Vaccin Immunother. 2013. A novel, disruptive vaccination technology: self-adjuvanted RNActive(®) vaccines.

Reporter mRNAs

Reporter genes are commonly used in cell biology research. Reporter mRNAs can be used as controls to study transfection and expression in mammalian cells using a variety of assays.

These capped (Cap 1) and polyadenylated mRNAs are optimized for mammalian systems and are composed of unmodified NTPs or are modified (moU replaces U) to reduce immune stimulation. They mimic fully processed mature mRNAs.

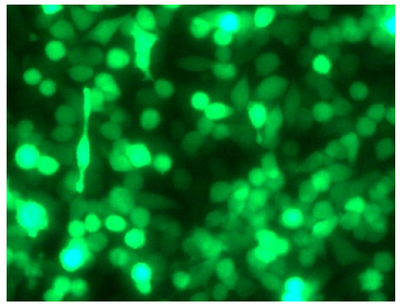


Figure 3 : Transfection of GFP mRNA with RmesFect on HeLa cells.

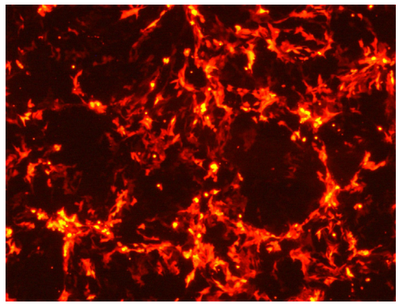


Figure 4 : Transfection of mCherry mRNA with RmesFect on SK6 cells.

OZ Biosciences mRNAs for Reporter mRNA :

TOMATO mRNA - ref# MRNA10 (5moU) - ref# MRNA9 (Unmodified) - ref# MRNA2 (N1-mψ)	Designed to produce high expression level of Orange Fluorescent Protein. The produced Tomato has an excitation maximum at 551-557 nm and emission maximum at 579-583 nm.
GFP mRNA - ref# MRNA11 (5moU) - ref# MRNA15 (Unmodified) - ref# MRNA22 (N1-mψ)	Designed to produce high expression level of Green Fluorescent Protein. It is a commonly used direct detection reporter in mammalian cell culture, yielding bright green fluorescence with an excitation peak at 488 nm and an emission peak at 507 nm.
F-Luc mRNA - ref# MRNA12 (5moU) - ref# MRNA16 (Unmodified) - ref# MRNA24 (N1-mψ)	Designed to produce high expression level of FireFly Luciferase. It is commonly used in mammalian cell culture to measure both gene expression and cell viability. FireFly Luciferase emits bioluminescence in the presence of the substrate, luciferin.
mCherry mRNA - ref# MRNA13 (5moU) - ref# MRNA8 (Unmodified) - ref# MRNA1 (N1-mψ)	Designed to produce the mCherry Fluorescent Protein which is derived from DsRed, a protein found in Discosoma sp. mCherry is a monomeric fluorophore with a excitation peak at 587 nm and emission at 610 nm. mCherry is photostable and resistant to photobleaching.
β-Gal mRNA - ref# MRNA14 (5moU) - ref# MRNA17 (Unmodified) - ref# MRNA13 (N1-mψ)	Designed to produce the enzyme encoded by the bacterial LacZ gene. Beta-Gal catalyzes the conversion of Beta-galactosides into monosaccharides. It is a common marker gene used to assess transfection efficiency by measuring enzymatic activity after X-Gal staining or colorimetric assay (CPRG, ONPG kit).
R-Luc mRNA - ref# MRNA21 (5moU) - ref# MRNA20 (Unmodified) - ref# MRNA7 (N1-mψ)	Designed to produce high expression level of Renilla Luciferase protein. R-Luc mRNAs can be used as control of transfection efficiency as a reporter gene.
Cy5 GFP mRNA - ref# MRNA11c (5moU) - ref# MRNA15c (Unmodified)	Designed to produce high expression level of Green Fluorescent Protein. It can be used as control of transfection efficiency.
Cy5 F-Luc mRNA - ref# MRNA12c (5moU) - ref# MRNA16c (Unmodified)	Designed to produce high expression level of FireFly Luciferase. It is commonly used in mammalian cell culture to measure both gene expression and cell viability. It emits bioluminescence in the presence of the substrate, luciferin.

Genome Editing mRNAs

Introduce a variety of genetic alterations (deletion, insertion...) into mammalian cells with Genome Editing techniques.

Genome Editing mRNA Benefits

Plasmids and viral vectors are traditionally used in genome editing to express the required proteins. mRNA-based strategy for delivery of CRISPR/Cas9 or CRE offers an advantage over pDNA-based approach:

- Cas9: Delivery of RNA encoding the Cas9 protein is an attractive non-viral method for introduction of the CRISPR/Cas9 machinery into the cell. Unlike gene-based delivery methods, mRNA-based stratgies are transient in function, leading to the eventual removal of the nuclease from the cell and circumventing the risks associated with integration into the host genome[9].

- Cre Recombinase: Site specific recombinases are useful tools for manipulation of genomes. However, continued expression of a recombinase in a cell or in vivo can result in toxicity and undesired off-target recombination. For this reason, transient expression from mRNA is an ideal method for recombinase expression.

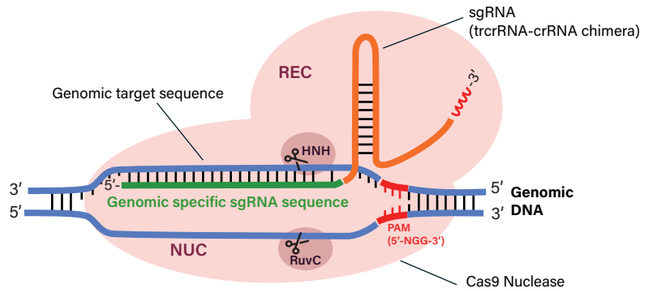


Figure 5 : The CRISPR-Cas9 nuclease programmed with sgRNA.

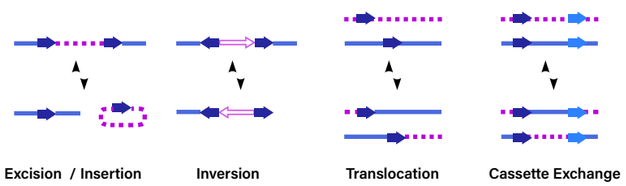


Figure 6 : Cre recombinase induces 4 types of site-specific genome modifictions. Excision/insertion - Inversion - Translocation - Cassette exchange (DNA is exchanged between two DNA molecules) Adapted from Nern A. et al, PNAS (108)34;14198–14203).

OZ Biosciences mRNAs for Genome Editing:

Cas9 Endonuclease mRNA - ref# MRNA31 (moU) - ref# MRNA30 (Unmodified) - ref# MRNA25 (N1-mψ)	The RNA-guided Cas9 endonuclease is used to induce site- directed double strand breaks in DNA. These breaks can lead to gene inactivation or introduction of heterologous genes, providing efficient tool for Genome Editing
CRE Recombinase mRNA - ref# MRNA32 (moU) - ref# MRNA33 (Unmodified) - ref# MRNA26 (N1-mψ)	Site-specific DNA recombinases are widely used in cells and organisms to manipulate the structure of genomes and to control gene expression by targeted activation or de-activation. Each recombinase catalyzes 4 types of DNA exchange reactions (Figure 8) between short specific target sequences (30-40 nucleotides).
Cas13d mRNA - ref# MRNA 28 (moU) - ref# MRNA 27 (Unmodified) - ref# MRNA 29 (N1-mψ)	Cas13 mRNA has been designed to produce high expression level of class 2 type VI-D CRISPR-Cas13d system derived from Ruminococcus flavefaciens XPD3002, a recently discovered RNA-guided RNA endonuclease.

9 Luther DC. et al, Exp. Opin Drug Deliv. 2018;15(9):905–913.

Other

Gene Replacement mRNA

EPO mRNA - ref# MRNA19 (5moU) - ref# MRNA18 (Unmodified) - ref# MRNA4 (N1-mψ)	This mRNA encodes for the human Erythropoietin protein, an hormone that controls erythropoiesis. EPO acts as a hematopoietic growth factor and stimulates the synthesis of red blood cells in the bone marrow. EPO mRNA is commonly used for **gene replacement** and serves as model for expression of any secreted protein. Its expression can easily be evaluated by enzyme-linked immunosorbent assay (ELISA) while EPO's effect on red blood cell production is detected by measuring reticulocyte levels and the hematocrit from whole blood using a hematocrit assay. Measurement of EPO are rather straightforward and well established.

Interleukin mRNA

h IL-2 mRNA - ref# MRNA56 (5moU) - ref# MRNA55 (Unmodified) - ref# MRNA57 (N1-mψ)	Designed to produce high expression level of Interleukin-2 (IL-2) proteins.

Custom service

With over 15 years of nucleic acid delivery expertise, OZ Biosciences is confident in providing you with the best-in-class service for the production of high quality mRNAs.

Features and Benefits

- **Custom-tailored service and support** to meet specific application or project needs.
- **A wide variety of modification options**: Concentration, buffer, modified nucleotides, desired UTR, with or without Cap and PolyA structure (ex.: moU pseudouridine mC).
- **Fluorescent mRNA labeling** with Cy5, Cy3 or other available options.
- **Flexible production scale** at microgram to multigram scales, from a few hundred up to several thousand bases.
- **High-quality mRNA synthesis**, in compliance with ISO 9001.
- **Competitive & affordable prices**.

Our service includes:

- Synthesis of the gene, cloning and DNA template production.
- mRNA synthesis by in vitro transcription.
- Purification and quality control.

All our mRNAs are purified and checked for their quality.

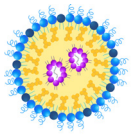
Our expert product support team is at your service for your special requests - you are invited to reach out to tech@ozbiosciences.com

OUR CUSTOM SERVICES

mRNA Synthesis

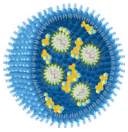
- Gene synthesis, Cloning & DNA template production.
- *In vitro* Transcription.
- Purification & Quality control.

NanOZ-LNP™ Design Platform



- Lipid Chemistry & Functionalization.
- Formulation Design & Manufacturing.
- NanOZ-LNPs™ Custom.

Customer DNA, RNA, API



- Provide us with your molecule of interest and we will formulate it into LNPs

BIOMEDICAL APPLICATIONS

Cancer Immunotherapy

Cell Programming

Vaccine

Gene Editing

Gene Therapy

Gene Silencing

OZ BIOSCIENCES
The art of delivery systems

USA & CANADA

OZ Biosciences USA Inc.
7975 Dunbrook Road,
Suite B,
San Diego, CA 92126
USA
Ph: +1 858 246 7840
Fax: +1 855 631 0626
orderusa@ozbiosciences.com

FRANCE & EUROPE

OZ Biosciences SAS
Parc Scientifique de Luminy
zone entreprise, case 922
13288 Marseille Cedex 09
FRANCE
Ph: +33 486 94 85 16
Fax: +33 486 94 85 15
order@ozbiosciences.com