# *Helix-IN™ Transfection Kit Results*

**Helix-IN** is the most powerful transfection reagent developed by OZ Biosciences. **Helix-IN** is based on the latest polymer-based transfection technologies that allow preserving membrane stability for higher viability while improving transfection efficiency. **Helix-IN** works in association with **HIB100X** designed to enhance overall transfection efficiency.

Main features are:

1. Broad spectrum transfection reagent for cell lines & difficult-to-transfect cells
2. Need of low amount of nucleic acid
3. Minimized toxicity & Low Cellular stress due to a new class of polymer-based reagent
4. High level of nucleic acid compaction
5. Increased protein production
6. Easy and straightforward protocol
7. Compatible with any culture medium

## Applications

**Helix-IN** has been developed for **DNA transfection** into a **broad spectrum of cells**; from classic cell lines to difficult-to-transfect cells. This polymer-based transfection reagent is serum compatible and can be used for transient as well as stable transfection. Helix-IN and HIB100X are very stable, ready-to-use and intended for research purpose only.

## Helix-IN™ Transfection efficiency - overview

Helix-IN is based on new polymeric tools and novel methods of synthesis that allows reaching high levels of efficiency with a lowered cellular stress and toxicity. Helix-IN was tested on various cell models and results demonstrated a significant high efficiency on both classic cell lines and difficult-to-transfect cells. Intensity of fluorescence (visible on fluorescent images) varies the same way than the % of efficiency.

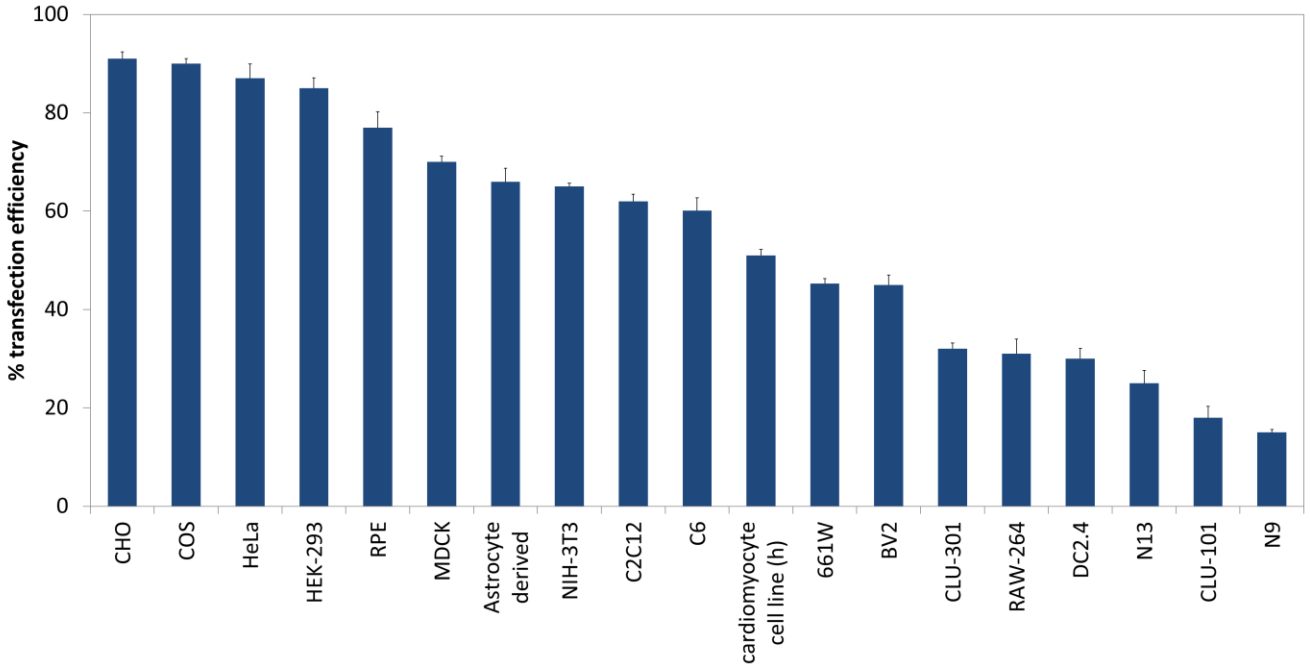


**Fig.1: Helix-IN + HIB100X were used to transfect 19 cell lines with a GFP-expression plasmid.** Transfections were performed in 24-well plate using 0.5 µg of pVectOZ-GFP (Ref #PL00020) and Helix-IN at a 2:1 ratio. HIB100X (1X final) was added to the complexes at the time of transfection and GFP expression was analysed 24 or 48H post transfection by cytometry analyses.

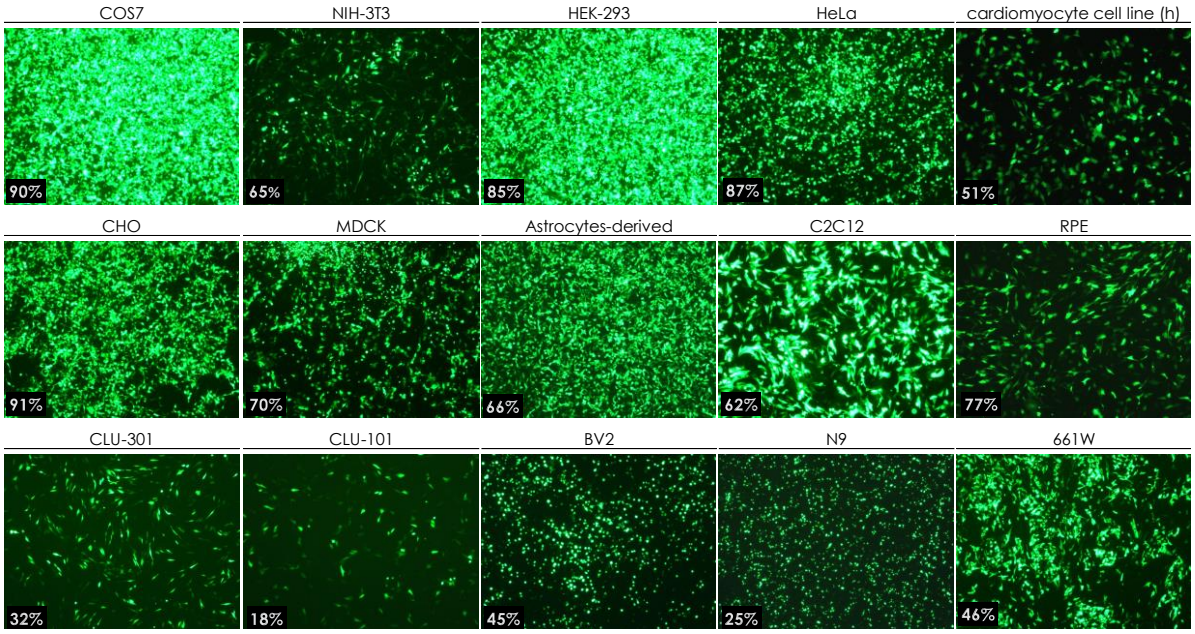


**Fig.2: Helix-IN + HIB100X transfection efficiency in cell lines.** Transfections were performed as described above and GFP expression was visualized under fluorescence microscopy 24 or 48H post-transfection.

## Optimization: time for the preparation of complexes

The standard protocol recommends incubating Helix-IN and nucleic acids at least 30 min to ensure the best transfection efficiency. Depending on the cell type, shorter periods of incubation may greatly influence either the overall % of transfection efficiency or the total amount of plasmid expression. Nevertheless, we do recommend incubating complexes at least 30 min before addition onto cells.

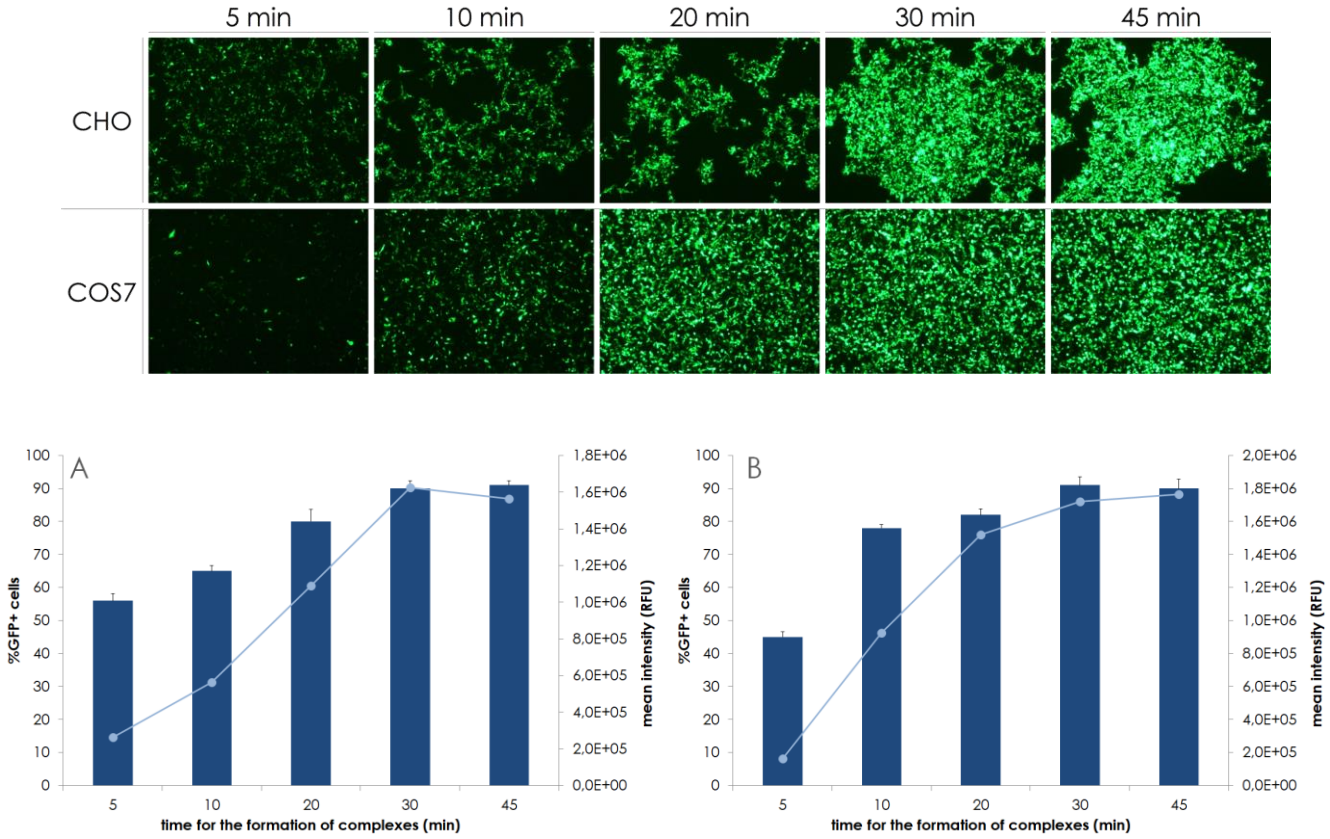


**Fig.3: Effect of the time for complexes formation on the transfection efficiency.** Complexes were prepared using Helix-IN and pVectOZ-GFP accordingly to the standard protocol (0.5µg, ratio 2:1) and incubated from 5 min to 45 min before addition onto the cells. 24 h after, the transfection efficiency of CHO-K1 and COS7 cells was evaluated by fluorescence microscopy and flow cytometry (graph A: CHO-K1, graph B: COS7); dark blue bars represent % of efficiency, clear blue line indicates median fluorescence intensity of transfected cells.

## Optimization: Matrix

In order to find the ideal DNA amount and Helix-IN ratio, optimization procedures were performed on cell lines. Best parameters were found to be 0.5µg of plasmid DNA in a 24-well plate format with a 1:1 ratio of Helix-IN. However, depending on the cell type, an optimization procedure maybe needed in order to find the best DNA amount and Helix-IN ratio.

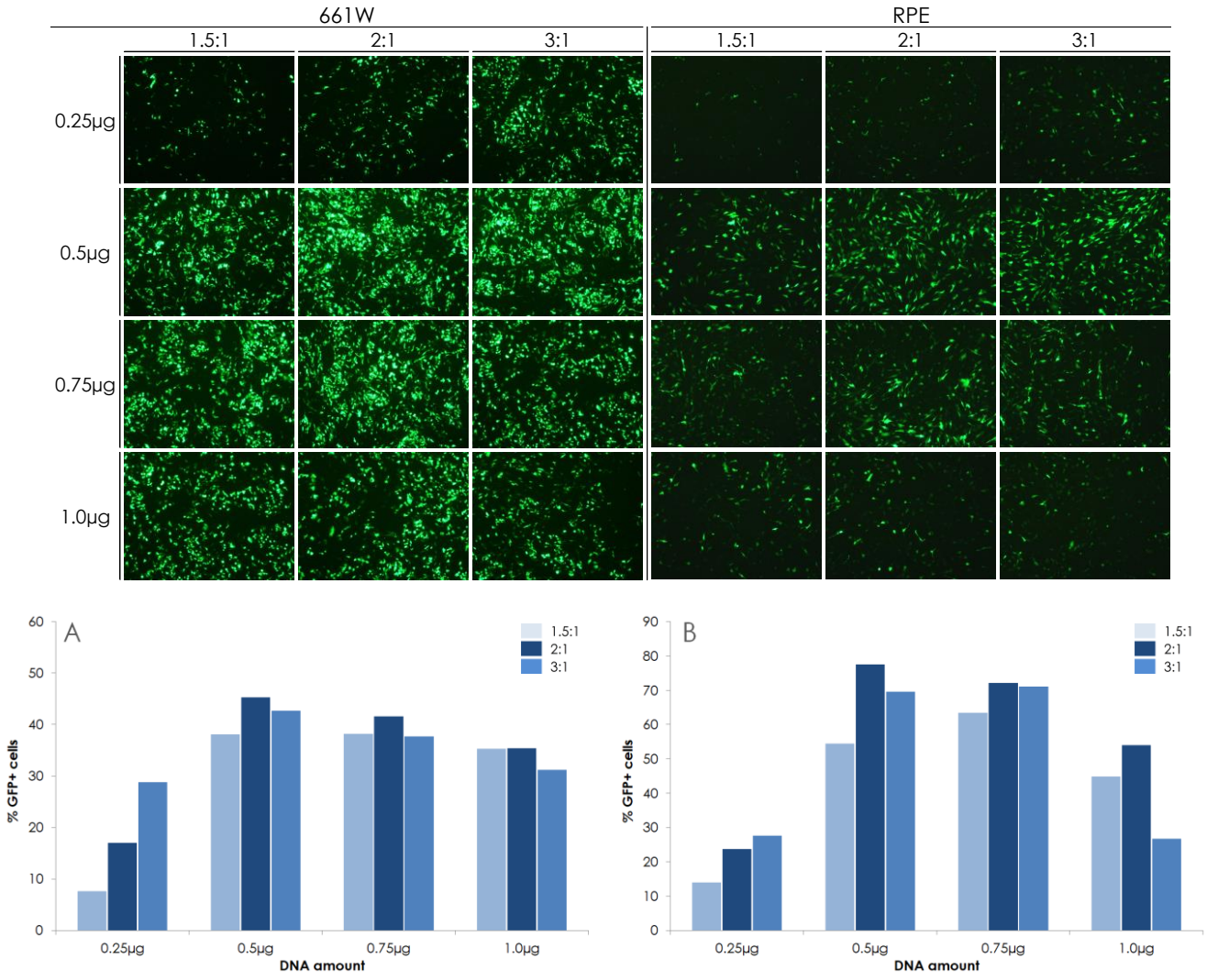


**Fig.4: Optimization of transfection conditions in 661W and RPE cell lines.** Complexes were prepared using 0.25 to 1µg/well of pVectOZ-GFP and 3 ratios of Helix-IN (1.5:1, 2:1 and 3:1, respectively 1.5µl, 2µL and 3µL per µg DNA). 24 h after, the transfection efficiency of 661W and RPE cells was evaluated by fluorescence microscopy and flow cytometry (graph A: 661W, graph B: RPE).

## Optimization: HIB100X Effect

**HIB100X was demonstrated to enhance transfection efficiency. Addition of HIB100X to Helix-IN/pVectOZ-GFP complexes at the time of transfection (1X final) not only increased the % of transfected cells, but most importantly improved the overall fluorescence intensity.**

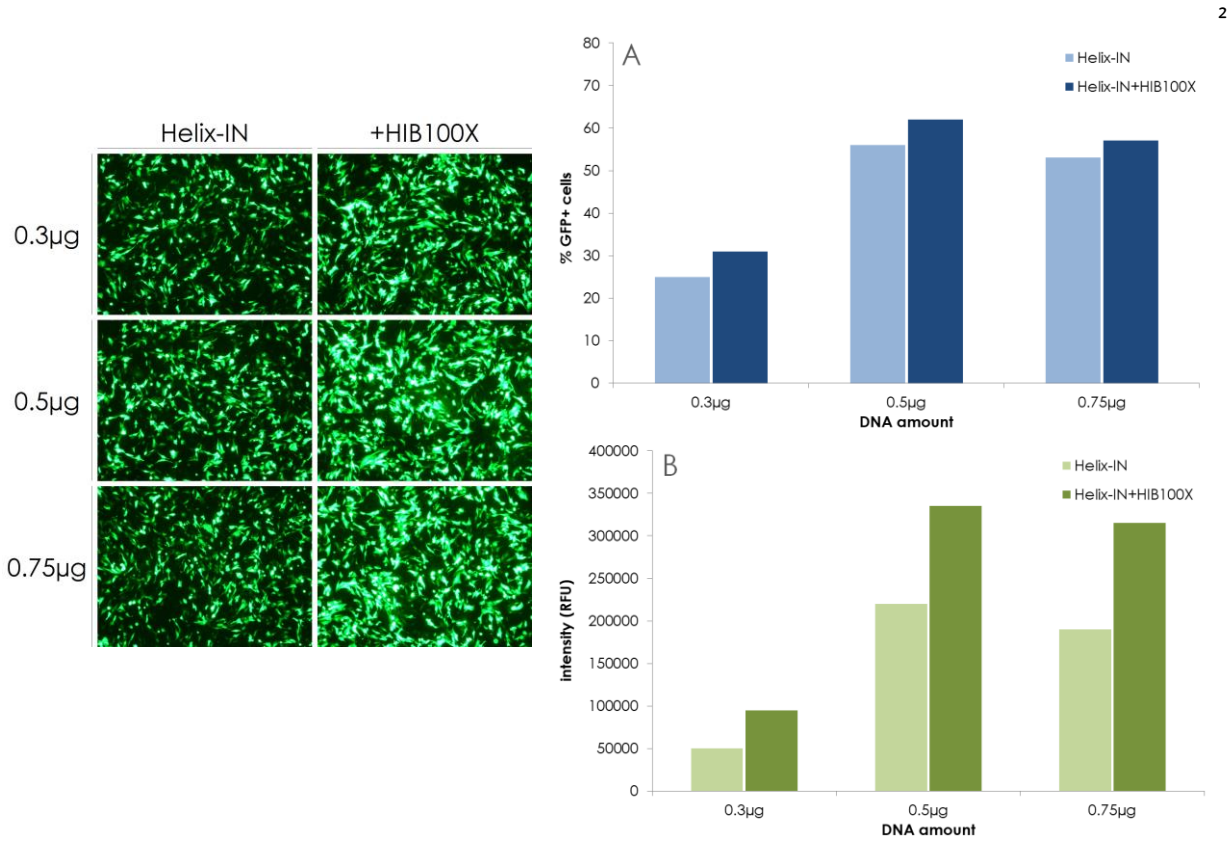


**Fig.5: HIB100X effect on C2C12 mediated Helix-IN transfection efficiency.** C2C12 cells were transfected with Helix-IN (ratio 2:1) complexed to 3 DNA amounts and HIB100X was added or not to the cells. 24H after transfection, efficiency was monitored by fluorescence microscopy and by flow cytometry. % GFP+ cells (A) shows that HIB100X have a positive effect on total % and median intensity measurement (B) confirms its role on gene production.

## Helix-IN™ and co-transfection

Helix-IN (+/-HIB100X) is greatly recommended for co-transfections as it allows (1) respecting stoichiometry of each plasmid composing the pool of vectors while (2) using very low doses of plasmid. Importantly even when one plasmid is used in excess, low quantities of the other are detectable in a dose dependant manner. Data demonstrated that highly linear expression of the two proteins occurs in close relationship to the dose of each plasmid transfected, respecting the stoichiometry of the transfection.

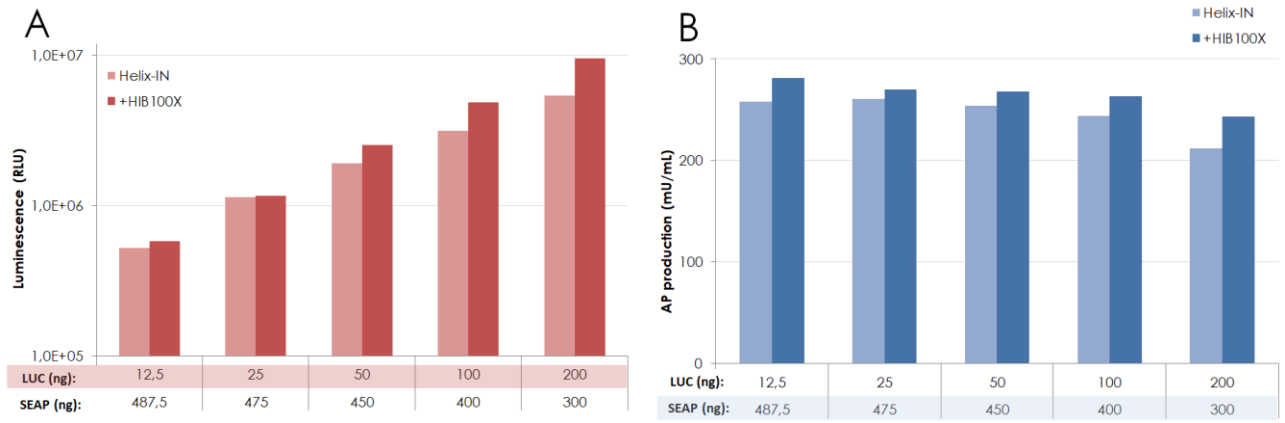


**Fig.6: Co-transfection efficiency using two plasmids with Helix-IN.** HEK-293 cells were co-transfected with various doses of pVectOZ-LUC (OZ Biosciences #PL00040) and pVectOZ-Seap plasmids (for a total DNA amount of 0.5µg). pVectOZ-LUC quantity was increased from 12.5ng to 200ng and mixed with decreasing amounts of pVectOZ-SEAP (from 487.5 to 300ng). Helix-IN was used at a 2:1 ratio and cell were transfected in presence or not of HIB100X. Luminescence and alkaline phosphatase production were monitored after 24H.

# *Helix-IN™ and Competitors*

Numerous assays were performed to compare Helix-IN to various competitors in terms of % of transfection efficiency, protein production – both extracellular and intracellular (respectively Secreted Embryonic Alkaline Phosphatase – SEAP, Luciferase and beta-galactosidase), toxicity and cellular stress. Results obtained demonstrated Helix-IN capacity to outperform competitors in most of the area; the new polymer-based technology makes Helix-IN the candidate of choice for any application.

## Helix-IN™ and competitors: Co-transfection

Helix-IN transfection reagent is ideal for co-transfection experiments. When compared to other commercial transfection reagents, Helix-IN presents a higher co-transfection rate with the same amount of DNA; this is mainly due to its high compaction level of nucleic acids: when complexed to Helix-IN, nucleic acids are protected from degradation until delivery into cells were endosome escape and nucleus targeting is facilitated.

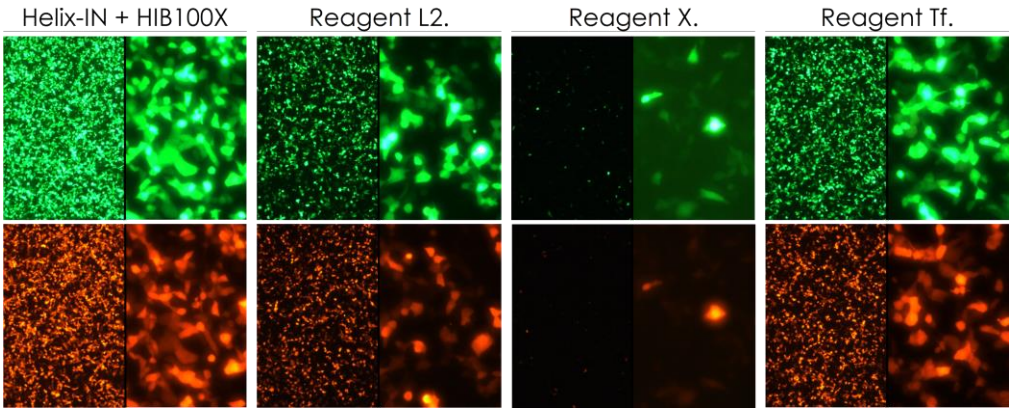


**Fig.7: Co-transfection efficiency using two fluorescent plasmids with Helix-IN.** HEK-293 cells were co-transfected with 0.25 µg or pVectOZ-GFP and 0.25µg of mCherry encoding vector (for a total DNA amount of 0.5µg) using Helix-IN (ratio 2:1) or commercial reagents following manufacturer's instruction. Green and Red protein expression were monitored under fluorescence microscopy 24H transfection to evaluate capacity to co-transfect cells with two plasmids (left side of photo: 4X magnification, right side of photo: 20X magnification).

## Helix-IN™ and competitors: Protein Production

Helix-IN outperforms competitors for protein production. Both secreted and intracellular protein production were evaluated in several types of cells: difficult-to-transfect and classic cell lines. 9 competitors were compared to Helix-IN. In every cases, best yield of protein were produced when using Helix-IN.

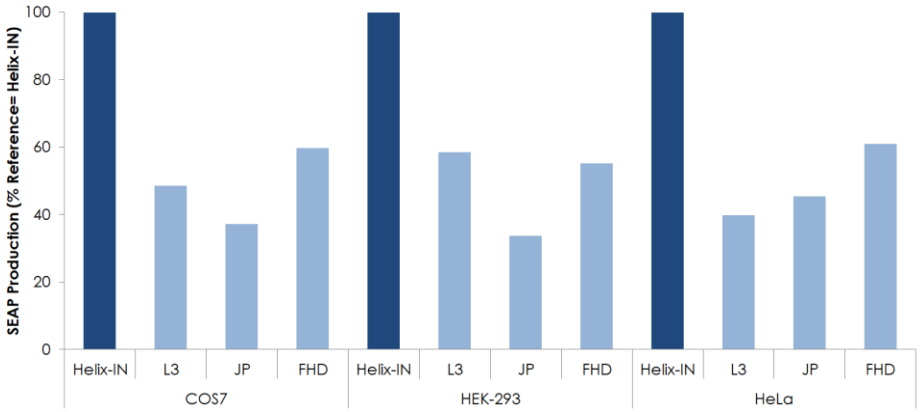


**Fig.8: Helix-IN vs competitors for extracellular protein production in classic cell lines.** COS7, HEK-293 and HeLa cell lines were transfected with Helix-IN+HIB100X and competitors according to their respective standard protocol using pVectOZ-SEAP (OZBiosciences, #PL00050). After 48H, 25 µL of supernatant were analysed using the SEAP assay Kit (OZBiosciences, #SP00500), Results are represented as a % of SEAP production in cells transfected with Helix-IN.

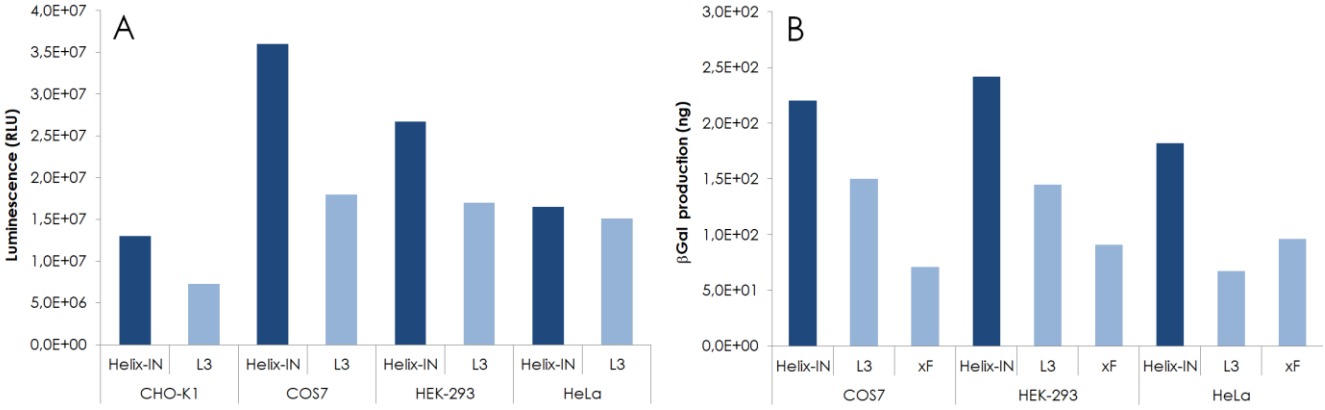


**Fig.9: Helix-IN vs competitors for intracellular protein production in classic cell lines.** CHO-K1, COS7, HEK-293 and HeLa cell lines were transfected with Helix-IN and competitors according to their respective standard protocol using (A) pVectOZ-LUC (OZBiosciences, #PL00040) or (B) pVectOZ-LacZ (OZBiosciences, #PL00030). 48H after transfection, emission of light and bGal production were measured.

## Helix-IN™ and competitors: Classic cell lines

Helix-IN outperforms the principal competitors in terms of transfection efficiency: both % of transfected cells and yield of fluorescence protein are superior to others when using Helix-IN reagent. The 5 main cell lines (CHO-K1, COS7, HEK-293, HeLa and NIH-3T3) were used to evaluate the transfection capacity of Helix-IN versus the 5 most cited-competitors.

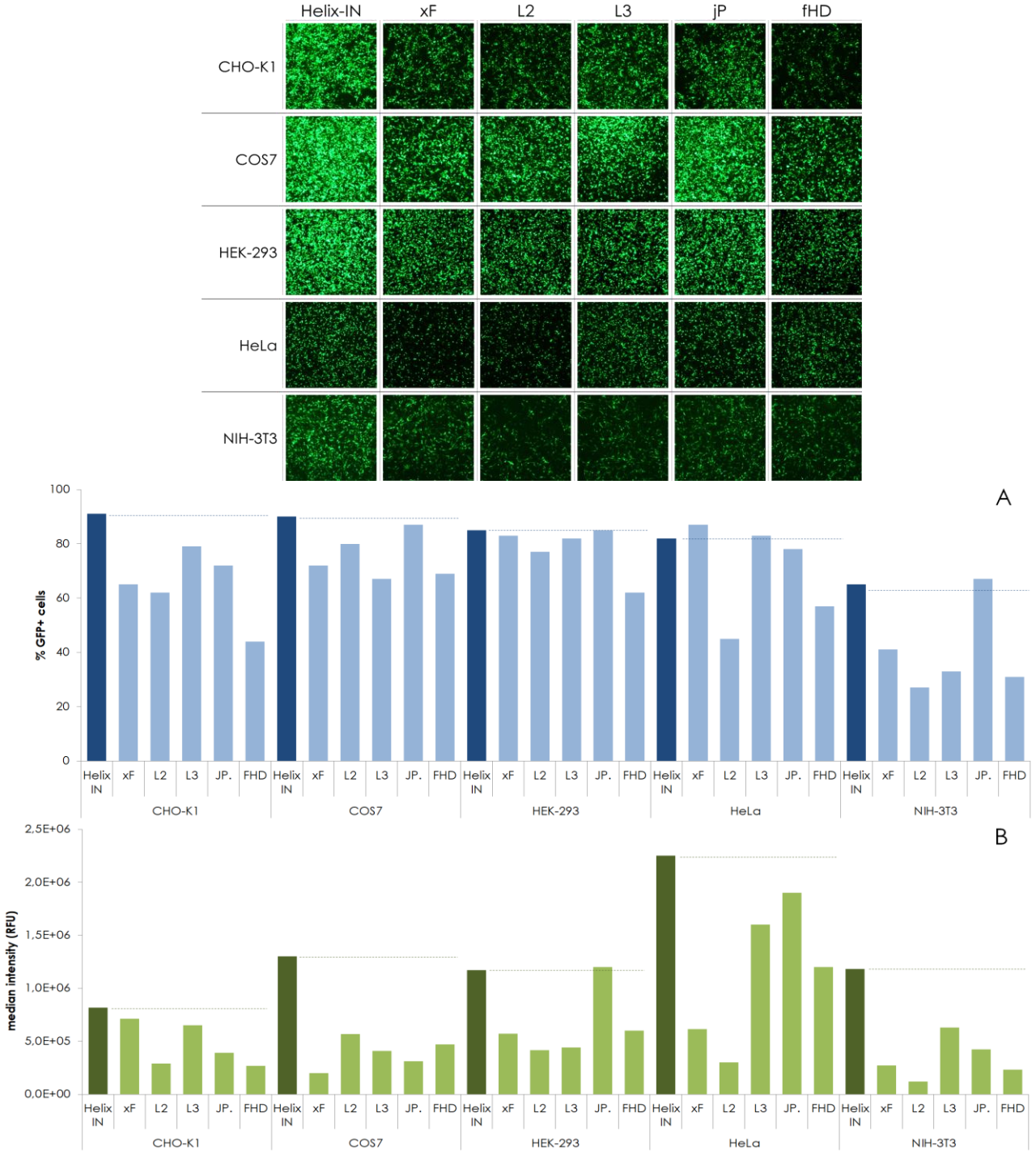


**Fig.10: Transfection efficiency in classic cell lines with Helix-IN compared to competitors.** CHO-K1, COS7, HEK-293, HeLa and NIH-3T3 cell lines were transfected with Helix-IN and competitors according to their respective standard protocol using pVectOZ-GFP (OZBiosciences, #PL00020). Transfection efficiency was monitored after 48H of incubation by fluorescence microscopy (upper image). % of GFP+ cells (A) and median fluorescence intensity (B) were measured using flow cytometry (lower graphs). Horizontal lines on both % and median intensity graphs represent the value of Helix-IN+HIB100X.

## Helix-IN™ and competitors: other cell lines

**Helix-IN was also compared to classic transfection reagents in 3 more difficult-to-transfect cells (C6, 661W and RPE).**

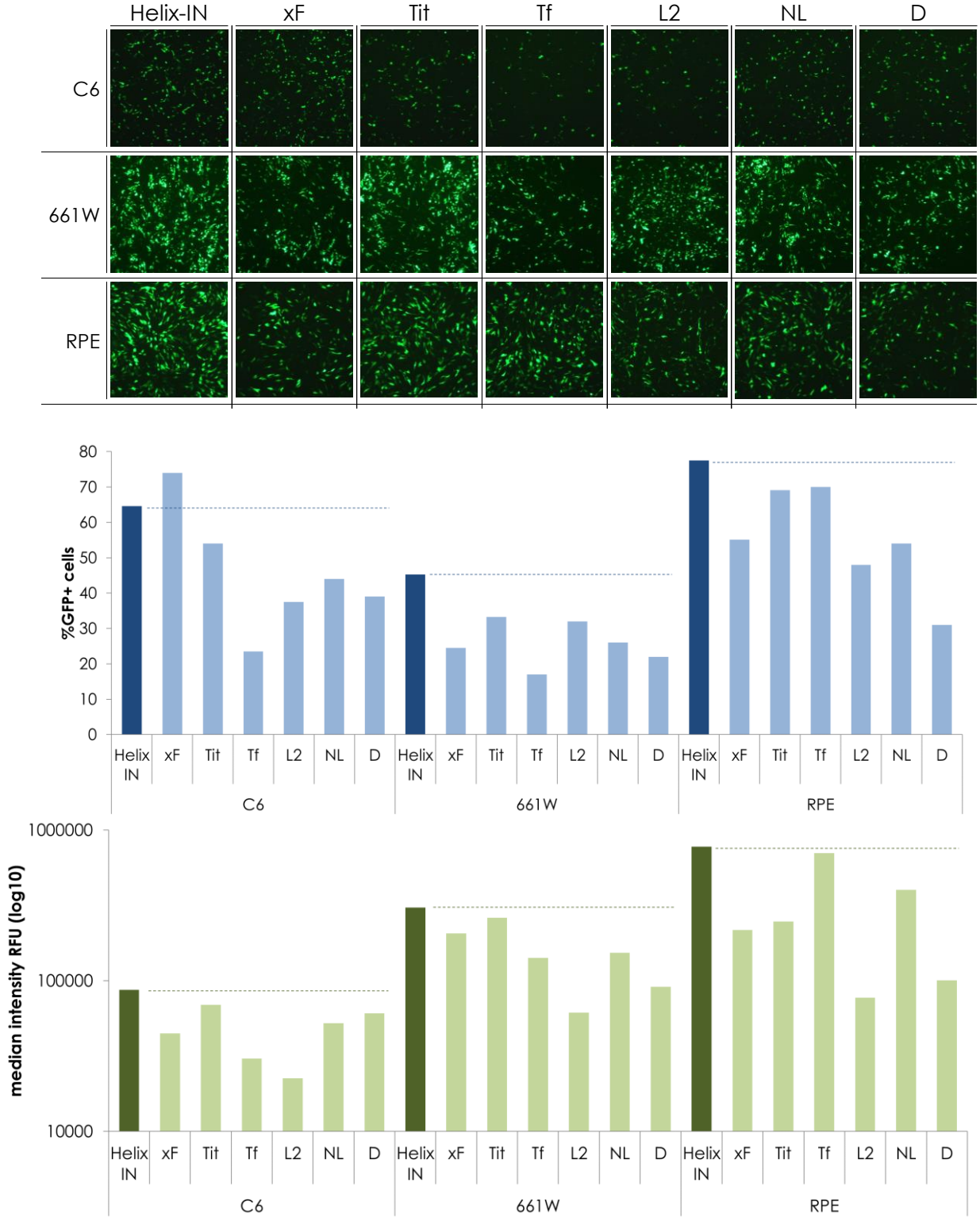


**Fig.11: Transfection efficiency other cell lines with Helix-IN compared to competitors.** C6, 661W, and RPE cell lines were transfected with Helix-IN and competitors according to their respective standard protocol using pVectOZ-GFP (OZBiosciences, #PL00020). Transfection efficiency was monitored after 48H of incubation by fluorescence microscopy (upper image). % of GFP+ cells (A) and median fluorescence intensity (B) were measured using flow

cytometry (lower graphs). Horizontal lines on both % and median intensity graphs represent the value of Helix-IN+HIB100X.

## Helix-IN™ and competitors: Protein Production and toxicity

An important feature of Helix-IN beside its capacity to transfect cells with high efficiency (considering both number of cells expressing the transgene and the yield of protein production), is represented by its high viability index. Especially when compared to principal competitors.

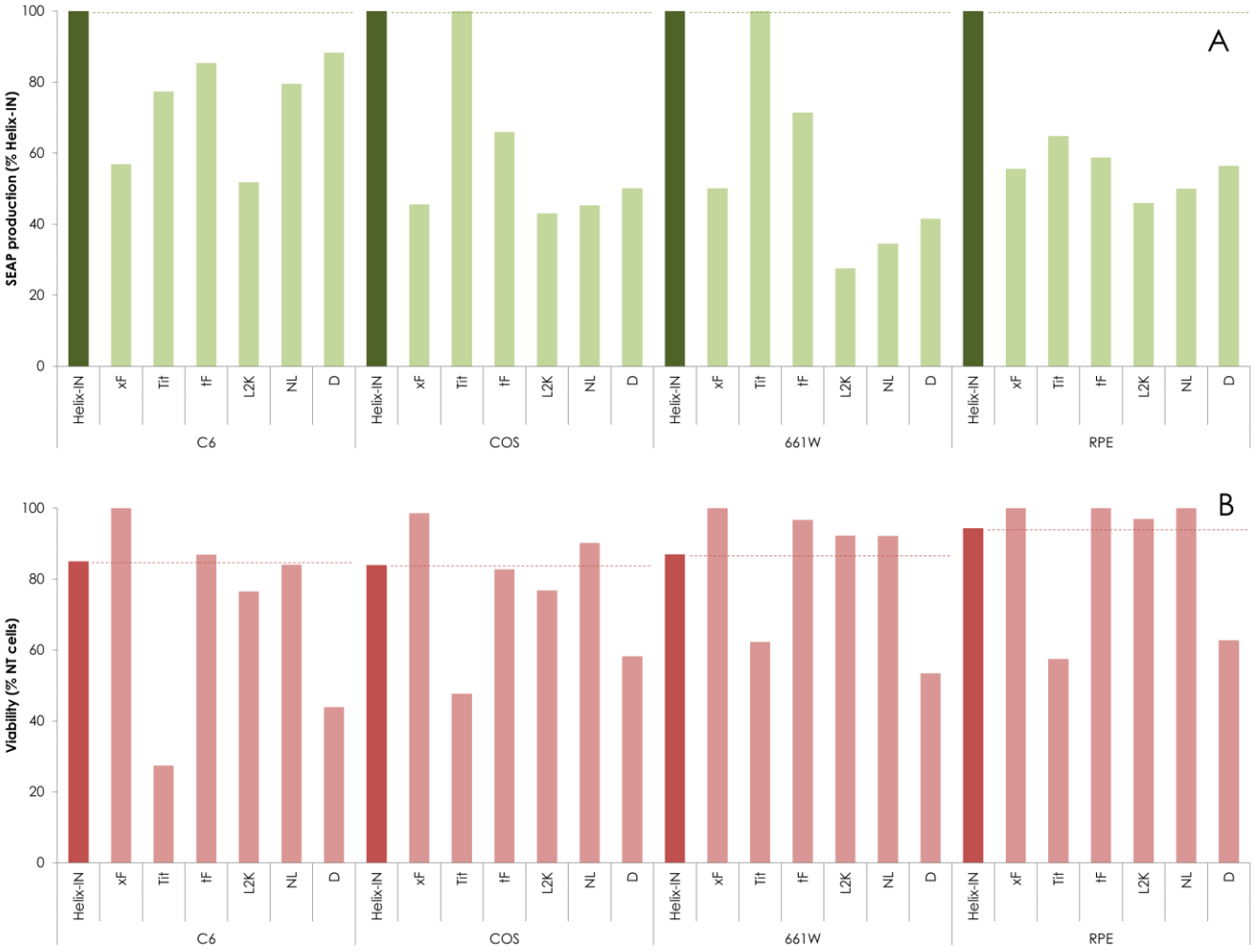


**Fig.12: Helix-IN vs competitors for secreted protein production and Toxicity.** C6, COS7, 661W and RPE cell lines were transfected with Helix-IN and competitors according to their respective standard protocol using pVectOZ-SEAP (OZBiosciences, #PL00050). 48H after transfection, 25 µL of supernatants were analysed using the SEAP assay Kit (OZBiosciences, #SP00500) and toxicity was evaluated on transfected cell monolayers using the MTT assay Kit (OBiosciences, #MT01000). Results are given as a % of SEAP production in cells transfected with Helix-IN and pVectOZ-SEAP (A) and as a % of viability compared to non-transfected cells (B).

## Helix-IN™ and competitors: cellular stress

**Helix-IN transfection kit allows to transfect cells with high efficiency while limiting the cellular stress.**

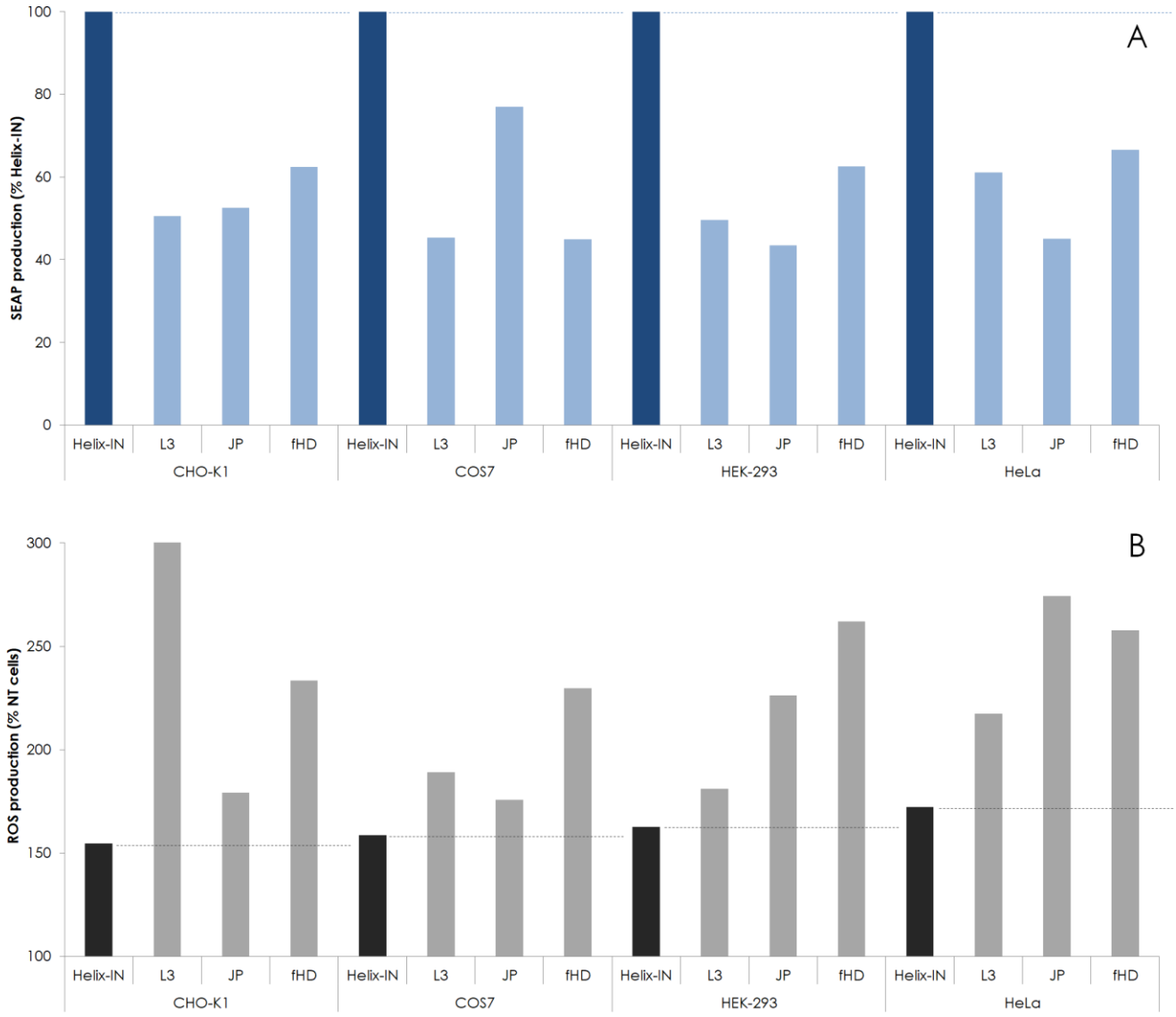


**Fig.13: Helix-IN vs competitors for secreted protein production and cellular stress.** CHO-K1, COS7, HEK-293 & HeLa cells were transfected with Helix-IN and competitors according to their respective standard protocol using pVectOZ-SEAP (OZBiosciences, #PL00050). 48H after transfection, 25 µL of supernatants were analysed using the SEAP assay Kit (OZBiosciences, #SP00500) and cellular stress was evaluated on transfected cell monolayers by monitoring ROS production using the ROS assay Kit (OBiosciences, #ROS0300). Results are given as a % of SEAP production in cells transfected with Helix-IN and pVectOZ-SEAP (A) and as a % of ROS production compared to non-transfected cells (B).