RmesFect™ - Results

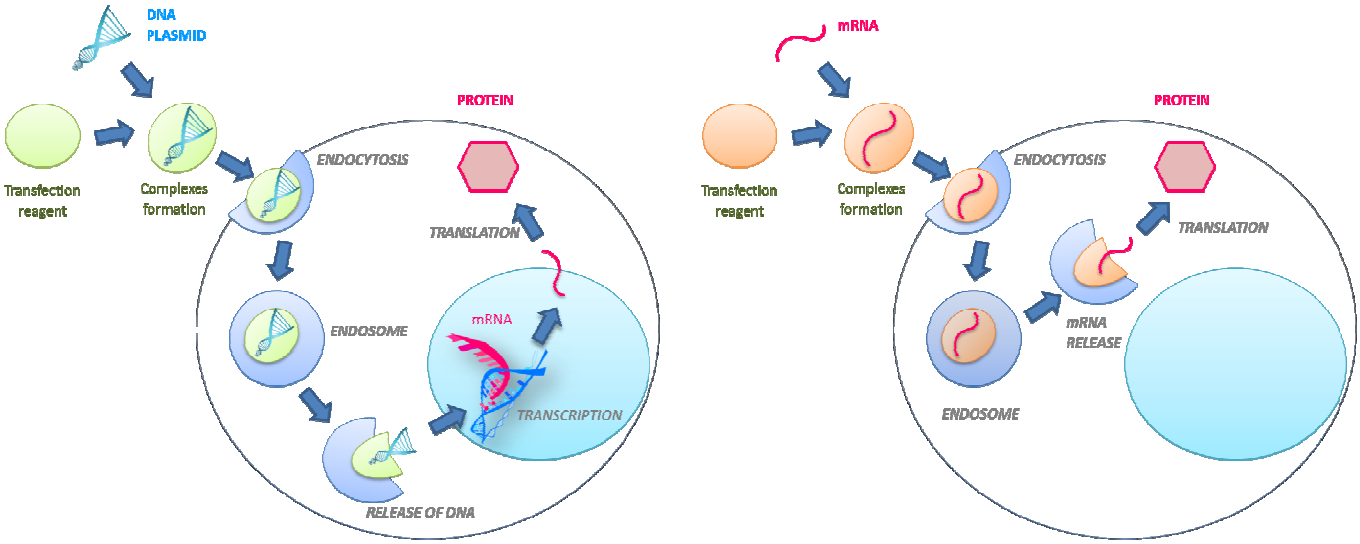
mRNA transfection provides two main advantages over plasmid DNA (pDNA) delivery. It does not require nuclear uptake for being expressed since translation of mRNA occurs into cytoplasm. Indeed, nuclear delivery (bypassing nuclear membrane) is one the principal barriers for transfecting slow or non-dividing cells and consequently, mRNA transfection is particularly attractive for such purpose. Moreover, this approach presents also the advantage of not being integrative. Contrary to pDNA, mRNA cannot lead to genetic insertion causing mutations.

Taking into consideration the nucleic acid types, size and function of messenger RNA, we have developed a new specific reagent allowing mRNA transfection with high efficiency. **RmesFect™** is based on the Tee-Technology ("Triggered Endosomal Escape") specifically designed for *in vitro* mRNA transfection in a large variety of cells. The cationic design of **RmesFect™** reagent allows to highly compacting mRNA for an efficient transport directly into the cytosol. In that way, nuclear delivery is no more required for gene expression to be effective.

RmesFect™ transfection reagent principal advantages:

- Highly efficient with all cells
- Ready-to-use: no need of additional buffer
- Low nucleic acid amount - minimized toxicity
- Protects mRNA against degradation
- Medium changed not required
- Easy and straightforward protocol
- Compatible with any culture medium

Applications



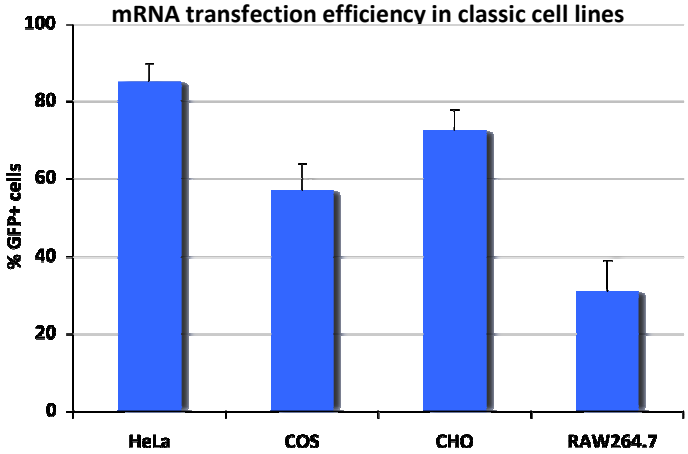
Transfection of mRNA with **RmesFect™** holds several benefits:

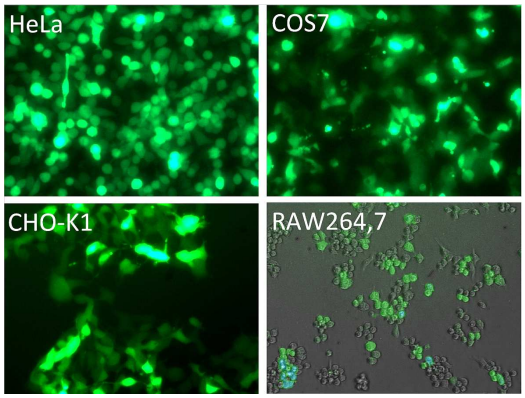
- No need of nuclear uptake - protein expression directly in cytoplasm
- Faster protein expression than DNA transfection
- No genomic integration
- Perfect for transfecting slowing or non-dividing cells
- Protein expression in a total promoter independent manner
- Transient transfection: mRNA based expression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

RmesFect™ has been developed for very efficient transfections of mRNA and dsRNA in a wide variety of immortalized and primary cells. This transfection reagent is serum compatible and is used for transient transfection. This product is very stable, ready-to-use and intended for research purpose only. mRNA transfection is particularly suited for mRNA vaccines, for cell reprogramming or IPs generation, primary cells transfection, regenerative medicine to name a few.

RmesFect™ transfection efficiency in cell lines

RmesFect™ transfection reagent is highly efficient for mRNA transfection in classical cell lines.
Complexes were prepared as followed: 0.5 µg of mRNA encoding GFP protein was mixed with RmesFect™ transfection reagent (ratio 2:1 for RAW264.7, 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. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.

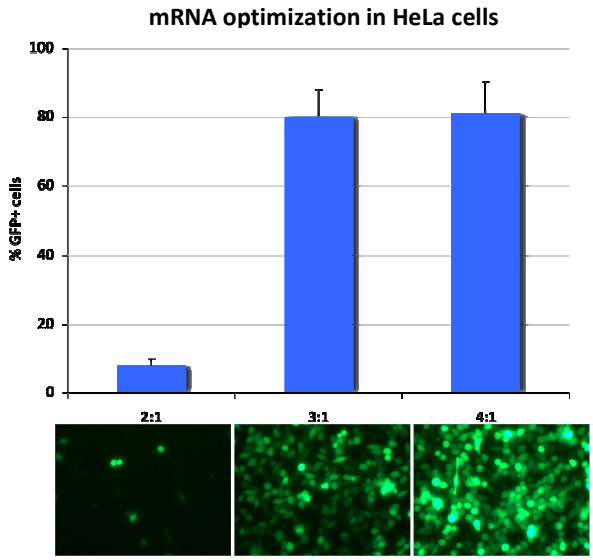


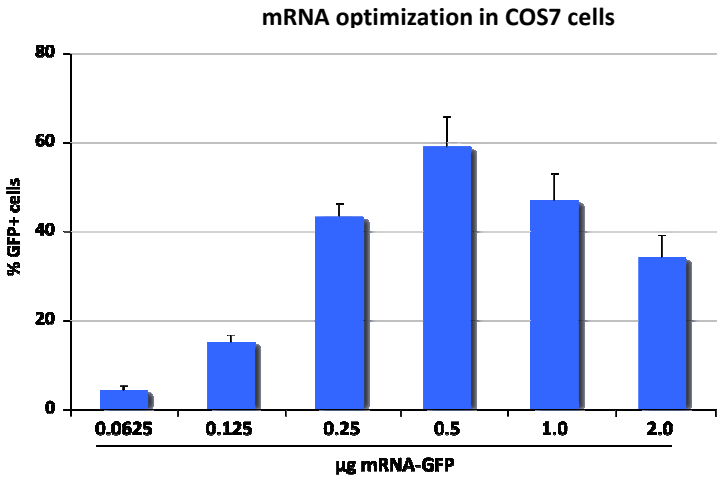


Results show that RmesFect™ transfection reagent is highly efficient in classic cell lines with low mRNA amount and reagent volume.
An updated list of successfully transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

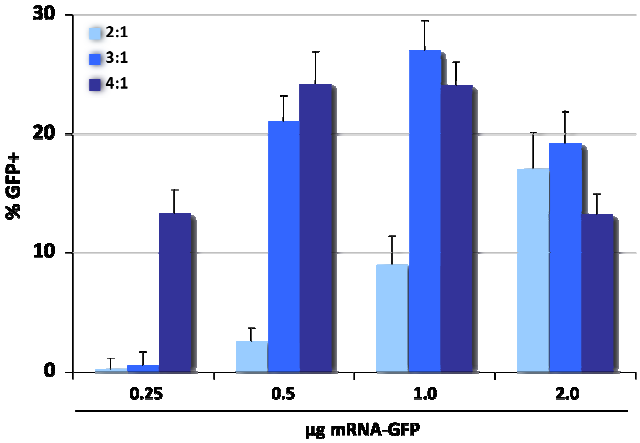
RmesFect™ optimization

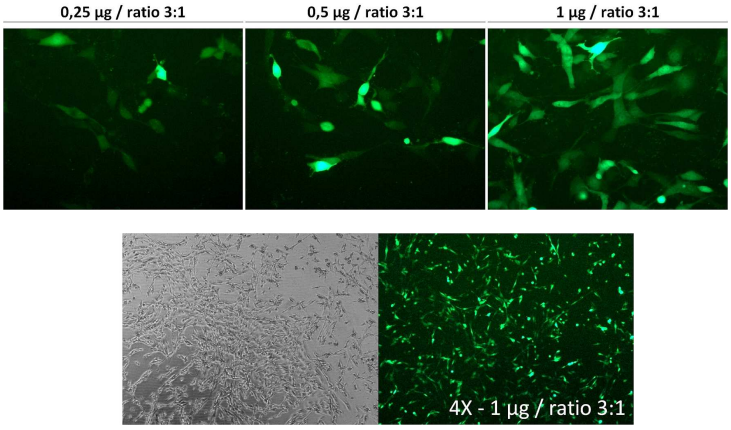
RmesFect™ optimization in cell lines.
For HeLa cells transfection, 0.5 µg mRNA were complexed with several volumes of RmesFect™ (corresponding to ratios 2:1, 3:1 and 4:1). For COS7 cell transfection, several quantities of mRNA were complexed with RmesFect at a 3:1 ratio. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.





Results highlight the optimization procedure of RmesFect in HeLa and COS7 cell lines.
For NIH-3T3, several quantities of mRNA were combined with several volumes of RmesFect™ corresponding to ratios 2:1, 3:1 and 4:1. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.



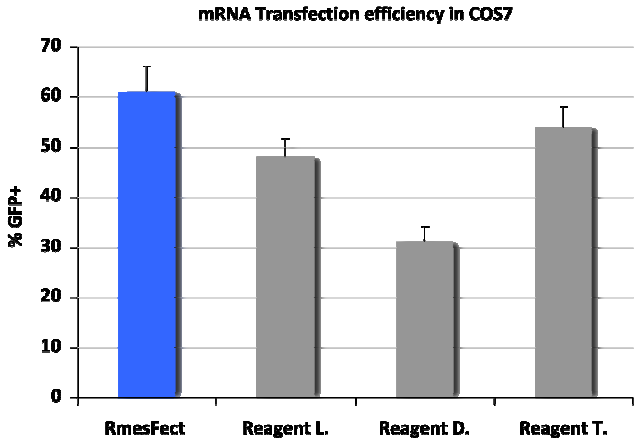


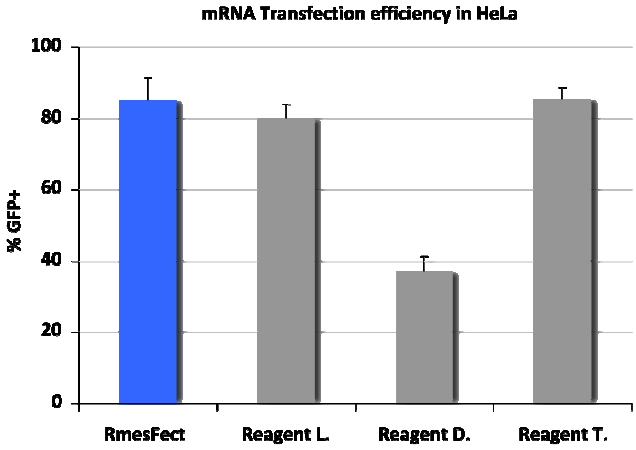
Results demonstrate the optimization procedure of RmesFect™ in NIH-3T3 cell lines.

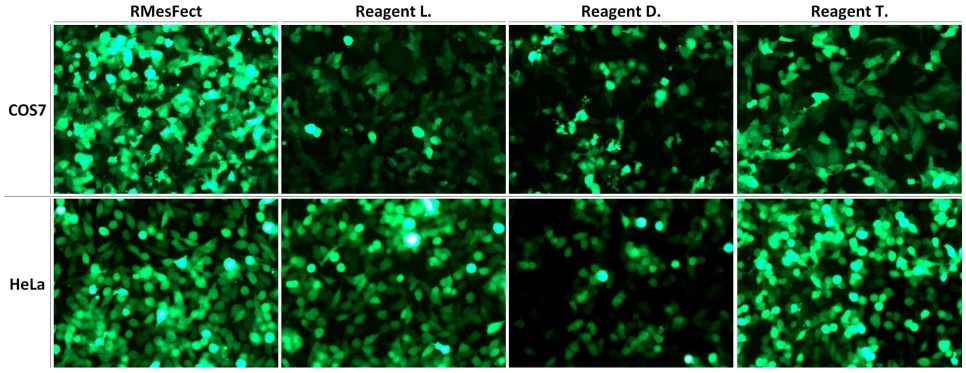
RmesFect™: comparison with other reagents

RmesFect™ transfection is highly efficient.

Complexes of mRNA and RmesFect™ were prepared as previously described and mRNA transfection with other commercial transfection reagents was performed as recommended by the manufacturers. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.





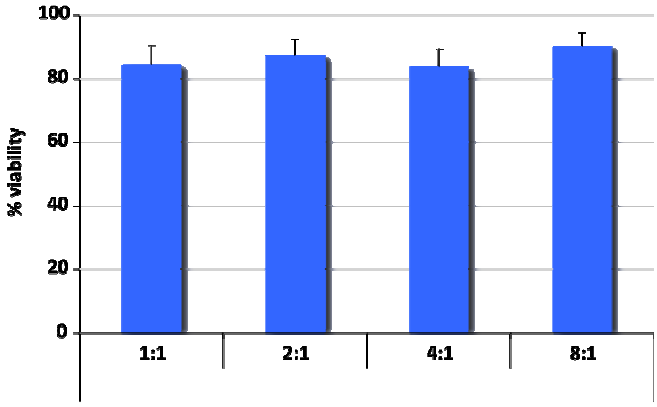


Results show that RmesFect™ transfection reagent allows transfecting cells with high efficiency when compared to other commercial transfection reagents.

RmesFect™ does not hamper cell viability

RmesFect™ transfection is non-toxic for the cells.
0.5 µg mRNA was complexed with several volumes of RmesFect (0.5, 1, 2 and 4µL) corresponding to ratio 1:1, 2:1, 4:1 and 8:1 respectively. After 24H transfection, MEF cells viability was measured with the MTT cell proliferation Assay Kit (OZ Biosciences - Ref # MT01000) and compared to un-treated cells.

Viability measurement in MEF cells.

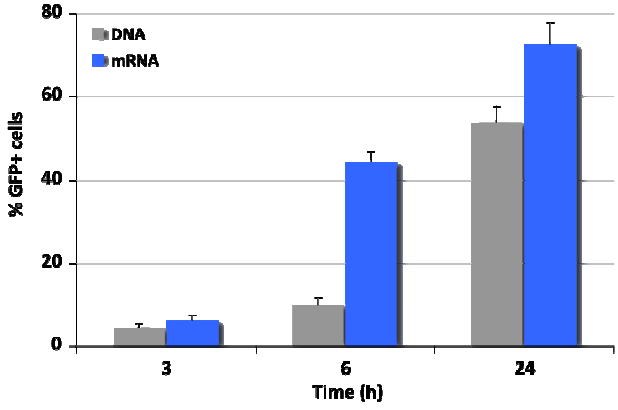


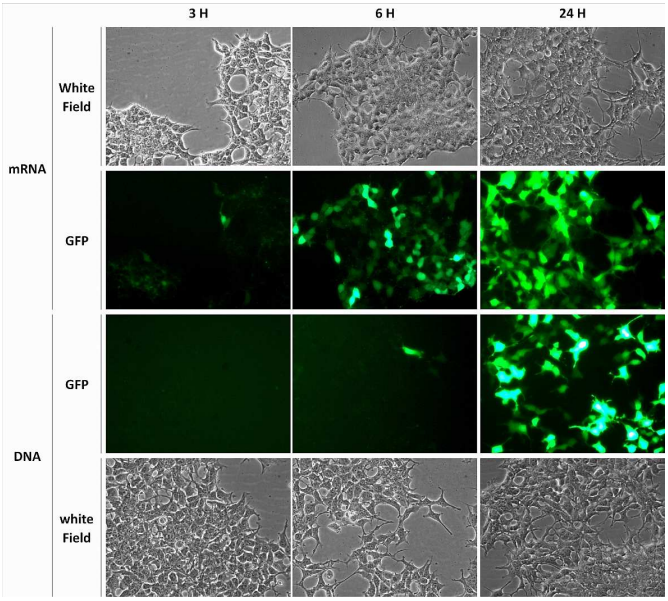
Results show that even when high ratios and volumes of RmesFect™ are used, viability is not hampered in the transfected cells.

Transfection of mRNA with RmesFect™ accelerates protein expression

RmesFect™ transfection reagent allows protein expression sooner than with DNA transfection.
Complexes of mRNA and RmesFect were prepared as previously described. For DNA transfection, 0.25 µg pVectOZ-GFP (OZ Biosciences - Ref #PL00120) were mixed with 0.75 µL of DreamFect Gold (OZ Biosciences). After 20 min of incubation at room temperature, complexes were added to the cells in a dropwise manner. 3H, 6H and 24 H after transfection, fluorescence expression in transfected cells was analysed by microscopy and FACS analysis.

Kinetics of GFP expression after mRNA and DNA transfection in CHO-K1 cells

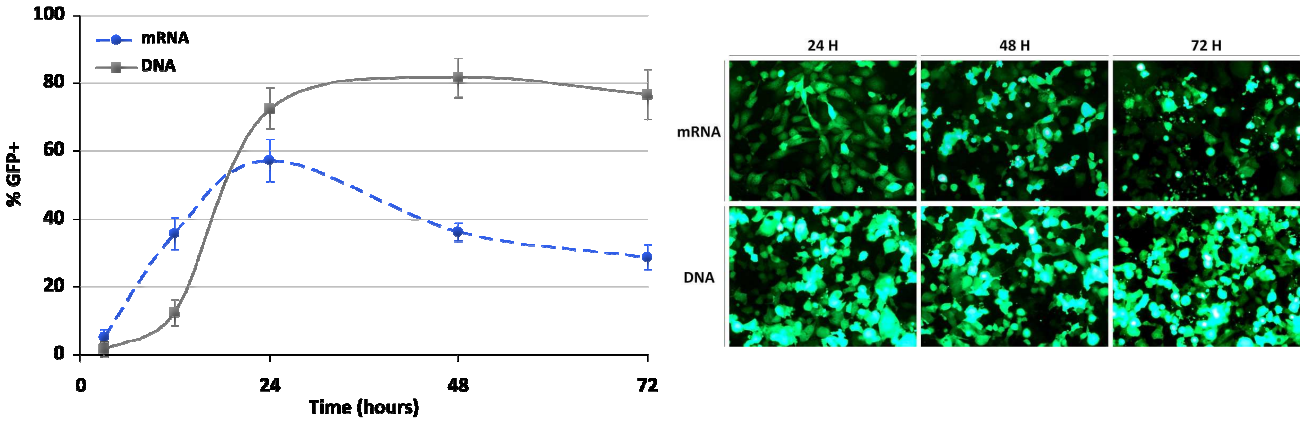




Results show that mRNA transfection with RmesFect allows protein expression sooner than with DNA transfection: mRNA transfection bypasses nuclear localization step enabling protein expression directly in the cytoplasm through transcription.

After 24H, protein expression resulting from mRNA transfection starts to decrease in contrast to plasmid DNA transfection. Complexes were prepared as previously described and % of GFP+ cells were determined 24, 48 and 72H after transfection by flow cytometry.

Kinetics of GFP expression after mRNA and DNA transfection in COS7 cells



Altogether the results show that mRNA transfection with RmesFect™ allows a protein expression sooner than with DNA transfection. Nevertheless, after 24H the protein production decreases while protein expression resulting from DNA transfection remains unchanged.

Our team has developed many cell type specific protocols with optimized transfection conditions. Please contact our technical support service to obtain these protocols: tech@ozbiosciences.com.

Bibliographic references

Please consult our list of references available on the website: www.ozbiosciences.com.