RmesFect™ Stem - Results

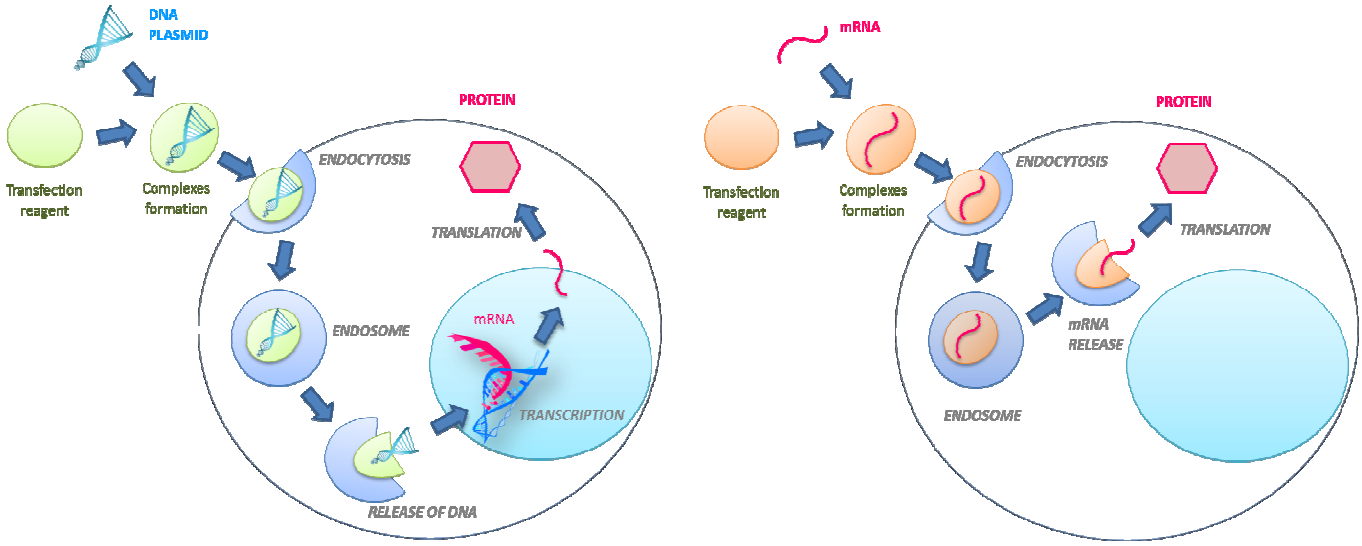
mRNA transfection provides two main advantages over plasmid DNA (pDNA) delivery. It does not require nuclear uptake for being expressed since translation of mRNA occurs in the cytoplasm. Indeed, nuclear delivery (bypassing the nuclear membrane) is one the principal barriers for transfecting slow or non-dividing cells and consequently, mRNA transfection is particularly attractive for such purpose. Moreover, this approach presents also the advantage of not being integrative which is particularly appealing for the stem cells field. 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 in stem cells. **RmesFect™ Stem** 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™ Stem** reagent allows high protection of mRNA for an efficient and gentle transport directly into the cytosol. In that way, nuclear delivery is no more required for gene expression to be effective.

RmesFect™ Stem transfection reagent principal advantages:

- Highly efficient with all cells
- Ready-to-use: no need for additional buffer
- Low mRNA amount - minimized toxicity- allowing daily transfections for iPS generation
- Protects mRNA against degradation
- Allows co-transfection of several mRNA
- Medium changed not required
- Easy and straightforward protocol
- Compatible with any culture medium

Applications



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

- No need for nuclear uptake - protein expression directly in the cytoplasm
- Faster protein expression than DNA transfection
- Faster cell reprogramming
- No genomic integration
- Protein expression directly related to mRNA quantity - precise control of various factors stoichiometry required during reprogramming
- Perfect for transfecting slow or non-dividing cells
- Protein expression in a total promoter-independent manner
- Transient transfection: mRNA based expression of proteins sustains for a limited time
- Allows RNA function studies

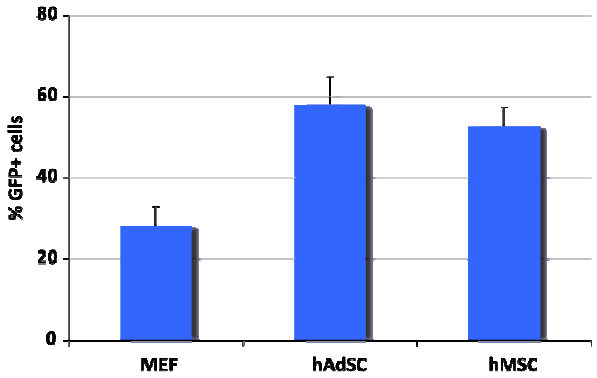
RmesFect™ Stem has been developed for very efficient transfections of mRNA and dsRNA in a variety of stem cells and primary cells. This transfection reagent is serum compatible and is used for transient transfection. This product is very stable, ready-to-use and intended for research purpose only. mRNA transfection is particularly suited for mRNA vaccines, primary cells transfection, regenerative medicine, cell reprogramming or for iPs generation. The latest normally requires 3–4 weeks to complete and necessitates repeated daily administration of mRNA due to their labile nature and short half-life. **RmesFect™ Stem** is the perfect reagent for such application thanks to its low toxicity and capacity to co-transfect several mRNA.

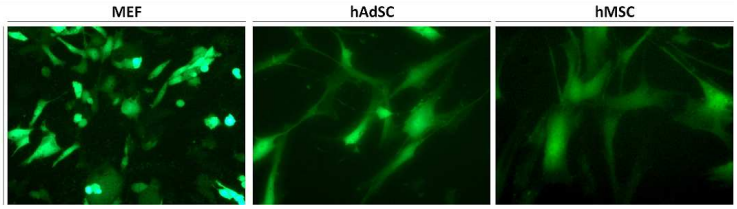
RmesFect Stem transfection efficiency in cells for reprogramming and Stem Cells

RmesFect™ Stem transfection reagent is highly efficient for mRNA transfection in cells used for cellular reprogramming and stem cells.

Complexes were prepared as followed: mRNA encoding GFP protein (0.25 µg for human Mesenchymal Stem cells and 0.5 µg for Mouse Embryonic Fibroblasts and human Adipose derived Stem Cells) was mixed with RmesFect Stem transfection reagent (ratio 2:1 for hMSC and 4:1 for MEF and hAdSC). After 20 min of incubation at room temperature, the complexes were added to the cells in a drop wise manner. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.

mRNA transfection efficiency in mouse embryonic fibroblasts and Stem Cells





mRNA transfection in :
MEF: mouse Embryonic Fibroblasts
hAdSC: human Adipose derived Stem Cells
hMSC: human Mesenchymal Stem Cells

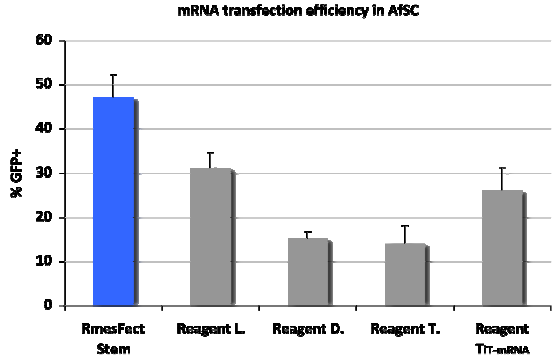
Results show that RmesFect Stem Transfection reagent is highly efficient in cells used for iPS generation such as in stem cells with low mRNA amount and reagent volume.

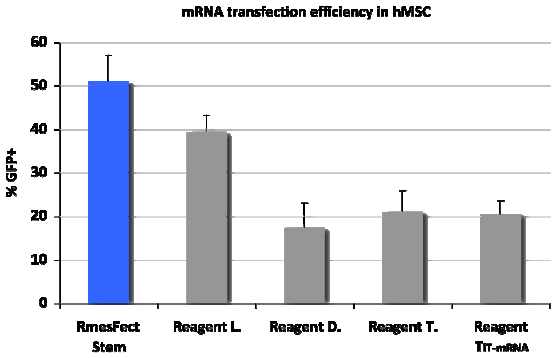
An updated list of successfully transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

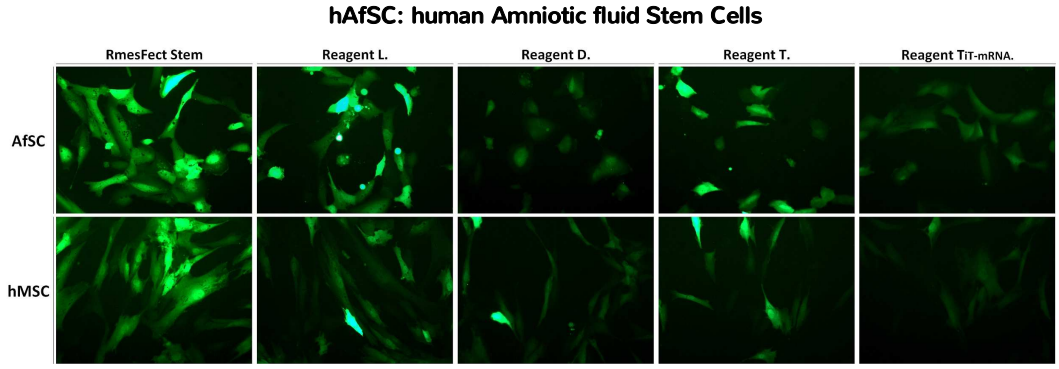
RmesFect™ Stem: outperforms other transfection reagents

RmesFect™ Stem 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.



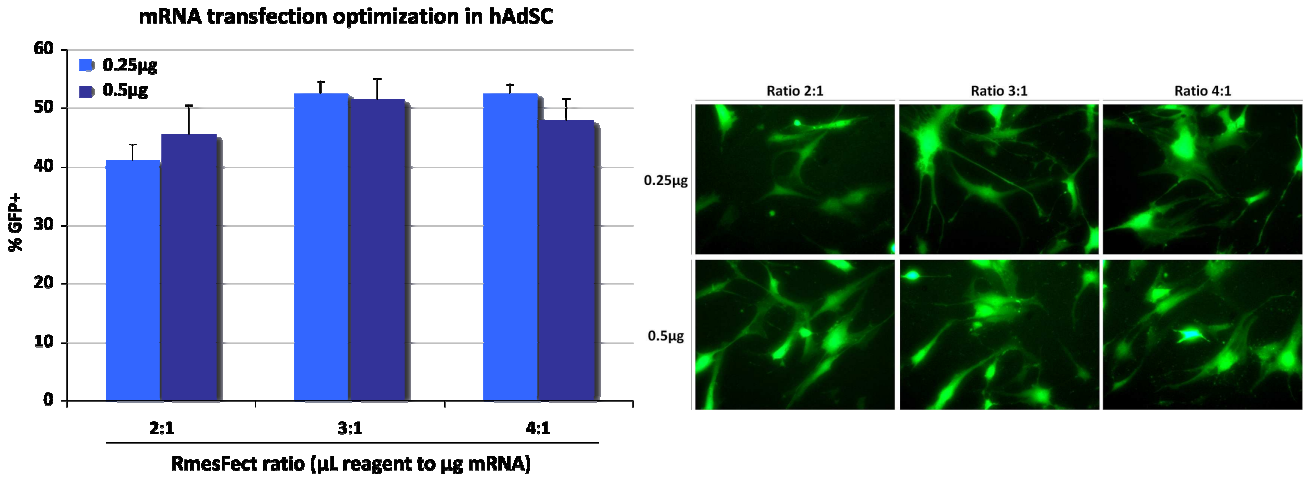




Results show that RmesFect™ Stem transfection reagent allows transfecting cells with higher efficiency than other commercial transfection reagents.

RmesFect Stem Optimization in hAdSC

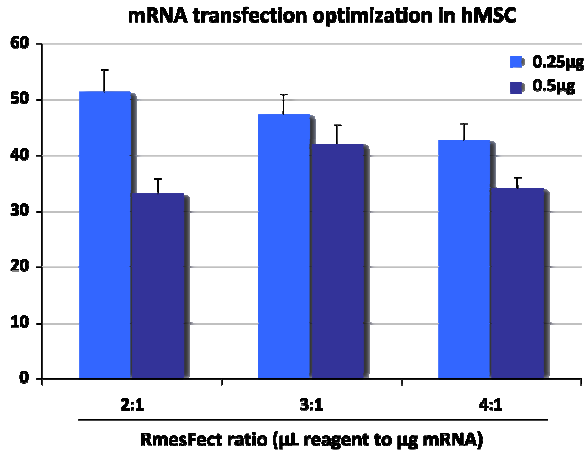
RMesFect Stem optimization in human Adipose derived Stem Cells (hAdSC).
0.25 and 0.5 µg mRNA were complexed with several volumes of RmesFect Stem (ratios 2:1 to 4:1) and used to transfect AdSC. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.

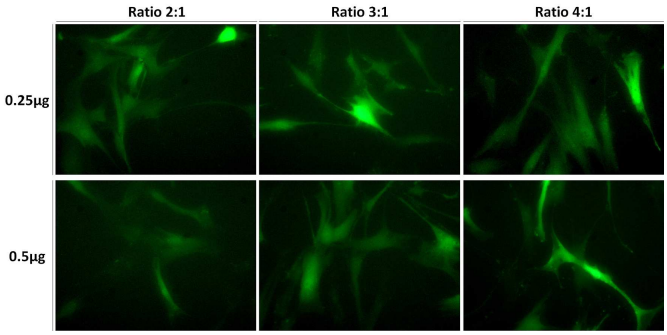


Results highlight the optimization procedure of RmesFect Stem in hAdSC, demonstrating the wide range of action of this transfection reagent in hAdSC.

RmesFect Stem Optimization in hMSC

RmesFect Stem optimization in human Mesenchymal Stem Cells (hMSC).
0.25 and 0.5 µg mRNA were complexed with several volumes of RmesFect Stem (ratios 2:1 to 4:1) and used to transfect hMSC. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.

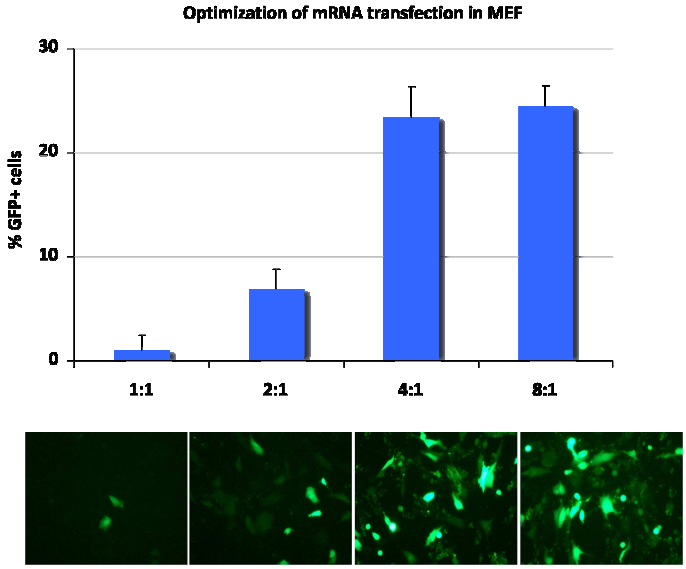




Results demonstrate the optimization procedure of RMesFect Stem in hMSC.

RmesFect Stem Optimization in MEF

RmesFect Stem optimization in mouse embryonic fibroblasts (MEF).
0.5 µg mRNA were complexed with several volumes of RmesFect Stem (corresponding to ratios 1:1 to 8:1) and used to transfect MEF. 24 H after, transfection efficiency was measured by fluorescence microscopy and FACS analysis.

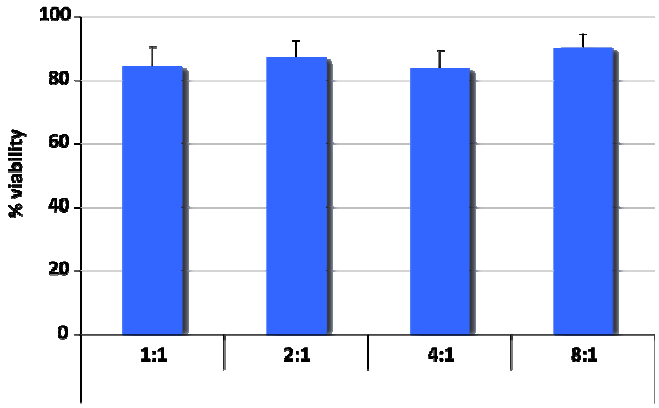


Results highlight the optimization procedure of RmesFect Stem in MEF.

RmesFect Stem does not hamper viability

RmesFect Stem transfection is non-toxic for the cells.
0.5 µg mRNA was complexed with several volumes of RmesFect Stem (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 (OZBiosciences - Ref # MT01000) and compared to un-treated cells.

Viability measurement in MEF cells.



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

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.