RmesFect™ Transfection Reagent

INSTRUCTION MANUAL

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RmesFect™

Instruction Manual

RmesFect™ is the newest transfection reagent specific for **mRNA** transfection.

List of RmesFect™ kits

Catalog Number	Description	Volume (µL)	Number of transfections with 1 µg of mRNA
KCR00500[1]	RmesFect™ Kit[1]	500 µL	125-250
RM20500	RmesFect™ transfection reagent	500 µL	125-250
RM21000	RmesFect™ transfection reagent	1 mL	250-500
RM25000	RmesFect™ transfection reagent	5 x 1 mL	1250-2500

[1] Contains one vial of RmesFect™ transfection reagent (500 µL) and 1 vial of mRNA encoding GFP protein (2.5 µg) as a positive control for transfection.

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (order@ozbiosciences.com). For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Technology

1.1. Description

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, 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 cell. The cationic design of **RmesFect™** reagent allows protecting mRNA from degradation for an efficient transport directly into the cytosol. In that way, nuclear delivery is no more required for gene expression to be effective.

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
- Allows RNA function studies

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

1.2. Kit Contents, Stability and Storage

Contents

Kits content varies according to their size:

- 1 tube containing 0.5 mL of RmesFect™ reagent good for up to 250 assays with 1 µg mRNA.
- 1 tube containing 1.0 mL of RmesFect™ reagent good for up to 500 assays with 1 µg mRNA.
- 5 x 1 tubes containing 1.0 mL of RmesFect™ reagent good for up to 2500 assays with 1 µg mRNA.
- The RmesFect™ Kit contains 1 tube of RmesFect™ (0.5 mL) and 1 tube of mRNA-GFP (2.5 µg).

Stability, Storage and Shipping

Storage: Upon reception and for long-term use, store the RmesFect™ transfection reagent at -20°C.
For the kit, mRNA-GFP positive control must be stored at -80°C.

RmesFect™ transfection reagent is very stable for several days at room temperature or +4°C without losing its activity. The storage at -20°C minimizes the size of liposomes and thus leads to higher efficiency. The numbers of freeze and thaw cycles do not affect the efficiency of the reagent.

Stability: RmesFect™ reagent and mRNA-GFP positive control are stable for at least 18 months at the recommended storage temperature.

Shipping condition: Room Temperature for RmesFect – Dry ice for mRNA-GFP positive control

2. Applications

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.

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.

3. General Protocol

3.1. General Considerations / Important Guidelines

The instructions given below represent sample protocols that were applied successfully with a variety of cells. Optimal conditions may vary depending on the mRNA, cell types, size of cell culture dishes and presence or absence of serum. Therefore, the amounts and ratio of the individual components may have to be adjusted to achieve best results. As a starting point, use **3 or 4 µL of RmesFect™ per µg of mRNA.**

- **Cells** should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The optimal confluence has to be adjusted according to the cells and the vessel used. We recommend using regularly passaged cells for transfection. Do not use cells that have been cultured for too long (> 2 months).

- **mRNA** should be as pure as possible, free of DNA, proteins, and other contaminants. Endotoxins levels must be very low since they interfere with transfection efficiencies.

- **Culture Medium**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the RmesFect™ transfection reagent.

3.2. Cells Preparation

Adherent cells protocol. It is recommended to plate the cells the day prior transfection in classical culture medium. Cells should be 70-80 % confluent at the time of transfection (see the suggested cell number in the Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and protein expression analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Suspension cells, co-transfection (DNA/mRNA) and reverse transfection protocols are also available. Please contact our technical support department (tech@ozbiosciences.com) for those specific procedures.

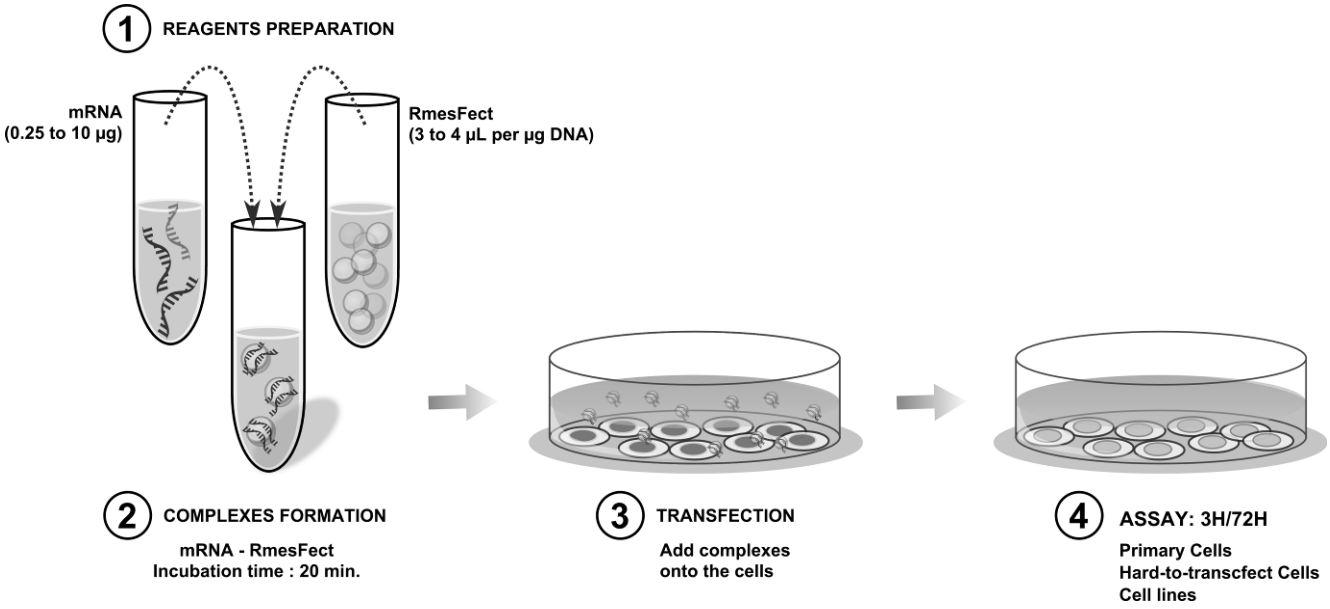
Table 1: Cell number suggested (per well).

Tissue Culture Dish format	Surface area per well[1]	Cell Number
96 wells	0.3 cm^2	0.05 – 0.2 x 10^5
24 wells	2 cm^2	0.5 – 1 x 10^5
12 wells	4 cm^2	1 – 2 x 10^5
6 wells	10 cm^2	2 – 5 x 10^5
60 mm	20 cm^2	5 – 10 x 10^5
100 mm	60 cm^2	10 – 30 x 10^5

[1] Surfaces area may vary depending on the manufacturer.

3.3. mRNA Transfection Protocol

Use the following procedure to transfect mRNA into mammalian cells. The Table 2 shows transfection conditions according to different cell culture formats (all amount are given on per-well basis). The mRNA and RmesFect solutions should have an ambient temperature and be gently vortexed prior to use. This rapid protocol, for most cell lines, is as simple as follows: use **3 or 4 µL of RmesFect™ per µg of mRNA**. We suggest beginning with the recommended ratios and optimize it, if required.



Cell culture prior to transfection. One day before transfection prepare the cells as indicated in 3.2

1) **Reagents preparation.** Allow reagent to reach room temperature before beginning.
   a. *mRNA solution.* Dilute 0.25 to 10 µg of mRNA in 25 to 1500 µL of culture medium without any supplement (SVF, antibiotics, growth factors...) or PBS (see Table 2). Depending on mRNA concentration, the proper quantity of mRNA can also be used directly without dilution.

   b. *RmesFect solution.* Mix the reagent gently before use. Dilute 0.75 to 40 µL of RmesFect™ in 25 to 1500 µL of culture medium without any supplement (SVF, antibiotics, growth factors...) or PBS (see Table 2).
   Note: If mRNA is used straightaway without dilution simply add the RmesFect solution to the mRNA tube.
   Note: Proceed quickly to step 2 to avoid any mRNA degradation or surface adsorption.

Table 2: mRNA amount, RmesFect volume and transfection conditions suggested (per well).

Tissue Culture Dish format	mRNA Quantity (µg)	RmesFect Volume (µL)	Dilution Volume (µL)[1]	Total culture medium Volume
96 wells	0.25	0.75 – 1.0	2 x 25	100 µL
24 wells	0.5	1.5 – 2	2 x 50	400 µL
12 wells	1.0	3 – 4	2 x 100	1 mL
6 wells	2.0	6 – 8	2 x 250	2 mL
60 mm	4.0	12 – 16	2 x 500	5 mL
100 mm	10.0	30 – 40	2 x 1500	15 mL

[1] Volumes of dilution medium for steps 1a and 1b.

2) **Complexes formation.** Combine the mRNA and the RmesFect solutions. Mix gently by carefully pipetting up and down and incubate the mixture for 20 minutes at room temperature. Do not vortex or centrifuge!

   Note: Proceed to step 3 within 30 minutes

3) **Transfection**. Add the complexes in a drop wise manner onto the cells growing in complete culture medium and homogenize by rocking the plate back and forth to ensure a uniform distribution of the mixture.

4) **Assay.** Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the protein expression. Depending on the cell type and mRNA activity, the assay can be performed as soon as 3 hours following transfection up to 72-96h. Generally, we recommend performing assay from 6 to 24h.

   Note: For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.

   Note: The medium can be changed after 4-6 hours of incubation with fresh medium for sensitive cells.

Reverse transfection. Reverse transfection can also be performed by plating the cells directly on top of the transfection mix. Prepare the complexes as described above, then transfer them into an empty culture dish or well and finally, directly add the cells at twice the recommended cell density.

Other protocols for suspension cells or co-transfection (mRNA/DNA) are also available on our website at www.ozbiosciences.com or by contacting our technical support department (tech@ozbiosciences.com).

OZ Biosciences offers two stable mRNA coding for GFP (#MRNA01-GFP) **and Luciferase** (#MRNA02-LUC) **as transfection controls**. . Use 1.5 or 2 µL of RmesFect with 0.5 µg mRNA (MRNA01-GFP or MRNA02-LUC) per well in a 24 well plate. These control mRNA are recommended to set up optimization procedure.

3.4. Optimization protocol for mRNA transfection

To achieve the highest efficiency, optimize the transfection conditions as follows:

- Vary the RmesFect (µL) / mRNA (µg) ratio from 1/1 to 6/1.
- Once the optimal mRNA/RmesFect ratio is found, adjust the mRNA quantity according to Table 3.
- Finally, culture medium compositions (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Table 3: Suggested range of mRNA amounts for optimization (per well).

Tissue Culture Dish format	mRNA Quantity (µg)
96 well	0.125 to 1
24 well	0.250 to 2
12 well	0.5 to 4
6 well	1 to 8
60 mm dish	2 to 16
100 mm dish	5 to 40

4. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com.

4.1 Quality Controls

To assure the performance of each lot of RmesFect™ reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 15 days.
Biological Activity	Transfection efficacies on CHO-K1 and COS7 cells. Every lot shall have an acceptance specification of > 85% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **Optimization of RmesFect / mRNA ratio.** See section 3.4. 2- **mRNA amount.** Use different quantities of mRNA with the optimized ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should be around 75% for mRNA transfection but most favorable cell density may vary according to the cell type; preferably mid-log growth phase. 4- **mRNA quality.** mRNA should be as pure as possible. Free of contaminants (DNA, proteins, phenol, ethanol etc.) and endotoxins. 5- **mRNA stability**. Ensure that mRNA is capped or polyadenylated to be protected from degradation. Polyadenylation and capping will increase stability and thus the level of expression. Alternatively, the internal ribosome entry site (IRES) from EMCV virus can be substituted for a 5' cap and increase the transduction. Stable mRNAs are available from OZ Biosciences as transfection controls 6- **Cell condition.** 1) Cells in culture for a long time (> 10 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing mRNA / transfection reagent complexes**. It is critical to use serum-free medium or buffer (HBS, PBS) during the complexes preparation. 8- **Culture medium composition.** 1) For some cells, transfection efficiency can be increased in absence of serum. Transfect these cells in serum-free medium during the first 4h. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 3h depending on the readout and the cell. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours. 10- **Old transfection reagent / mRNA complexes.** The transfection reagent / mRNA complexes must be freshly prepared each time to avoid aggregation. 11- **Transfection reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials. 2- **Protein expression is toxic.** Use suitable controls such as cells alone, transfection reagent

	alone or mock transfection with a mRNA control. 3- **mRNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 5- **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(for retrovirus and lentivirus)* / AdenoMag *(for adenovirus and aav)*
***In vivo* Targeted Delivery**
In vivo PolyMag (*for all nucleic acids*)
In vivo ViroMag *(for all viral vectors)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
FlyFectin *(for Insect cells)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)*
Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ, Luc, CAT, GFP, SEAP
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
Stabilized mRNAs
Genome Editing mRNAs, Vaccine mRNAs, Reprogramming mRNAs, Reporter Gene mRNAs

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. http://www.ozbiosciences.com.

Purchaser Notification

Limited License

The purchase of the RmesFect™ reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). Reagents are intended **for in-house research only** by the buyer. Such use is limited to transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

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Buyers may end this License at any time by returning all RmesFect reagent material and documentation to OZ Biosciences, or by destroying all RmesFect™ reagent components. Purchasers are advised to contact OZ Biosciences with the notification that a RmesFect™ reagent kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

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Product Use Limitations

The RmesFect™ reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

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