Transfection reagent

RmesFect™

Tee Technology (Triggered Endosomal Escape)

Designed for mRNA tra.nsfection

Protocol

We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC

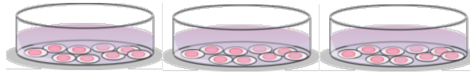
OZBIOSCIENCES

The art of delivery systems

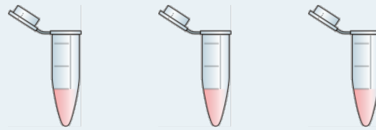
RmesFect™ Quick Protocol

To find the ideal conditions, RmesFect™ must be tested at ratios **2 µL/µg**, **3 µL/µg** and **4 µL/µg** (µL of RmesFect / µg of mRNA). For the mRNA quantity, we suggest **0.25 µg** per well in 96-well, **0.5 µg** per well in 24-well and **2 µg** per well in 6-well.

1 **Seed cells to be at 50-70% confluent the day of transfection***

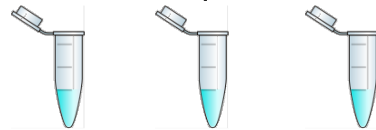


2 **Prepare 3 identical tubes of mRNA**



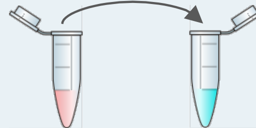
96 well plate	24 well plate	6 well plate
0.25µg in 25µL of serum-free medium or buffer* X 3	0.5µg in 50µL of serum-free medium or buffer* x 3	2µg in 100µL of serum-free medium or buffer* x 3

3 **Prepare 3 tubes of RmesFect™ (with 3 different amounts of reagent)**

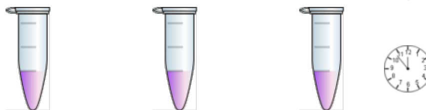


96 well plate	24 well plate	6 well plate
0.5µL/0.75µL/1µL in 25µL of serum-free medium or buffer*	1µL/1.5µL/2µL in 50µL of serum-free medium or buffer*	4µL/6µL/8µL in 100µL of serum-free medium or buffer*

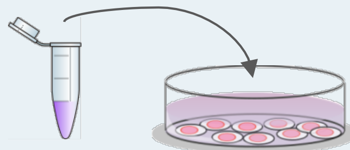
4 **Mix each tube of mRNA (step 2) to each tube of RmesFect™ (step 3)**



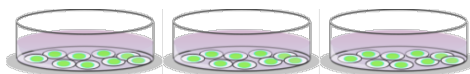
5 **Incubate 20 min at room temperature**



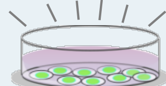
6 **Distribute each mix dropwise onto the cells to insure uniform distribution**



7 **Incubate cells for 24 to 72h at 37°C until evaluation of mRNA expression**



8 **Choose the best ratio mRNA:RmesFect™**



These conditions might require some further optimizations depending on your cells, RNA, read-out, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.

- ✓ 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).

- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.

- ✓ **Medium or buffer without serum & supplement** must be used for the DNA/RmesFect complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.

- ✓ Dilute the reagent with deionized water for doses less than 1µL.

- ✓ For some cells, 24 hours post-transfection replace the medium with fresh pre-warm medium or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be replaced after 3-4 hours.

Package content	RM20500: 500µL of RmesFect RM21000: 1mL of RmesFect RM25000: 5 x 1mL of RmesFect
Shipping conditions	Room Temperature
Storage conditions	Store the RmesFect transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	RmesFect is a lipopolyamine formulation specifically designed for mRNA transfection with high efficiency and low toxicity.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells preparation

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 (refer to 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.

Tissue Culture Dish	Cell Number
96 wells	$0.05 – 0.2 \times 10^5$
24 wells	$0.5 – 1 \times 10^5$
6 wells	$2 – 5 \times 10^5$

Table 1: Suggested cell number (per well)

2. mRNA/RmesFect complexes preparation

a. *mRNA solution*. Dilute the indicated quantity of mRNA in 25 to 250 µL of culture medium without any supplement (refer to Table 2).

Tissue Culture Dish	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
6 wells	2.0	6 - 8	2 x 250	2 mL

[1] Volumes of dilution medium for steps 2a and 2b

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

NOTE: 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 the indicated quantity of RmesFect in 25 to 250 µL of culture medium without any supplement (see Table 2).

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

IMPORTANT NOTES

- Proceed quickly to complex formation to avoid any mRNA degradation or surface adsorption.
- Proceed to transfection within 30 minutes.

3. Transfection

a. 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.

b. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the protein expression.

NOTES:

- 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.
- In case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.
- Reverse transfection can also be performed: add complexes first, and then add cells at twice the recommended cell density.

Protocol | mRNA transfection in suspension cells

1. Cell Preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium (refer to Table 3).

Tissue Culture Dish	Suspension Cell Number	mRNA Quantity (µg)	RmesFect Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.5 – 1 x 10^5	0.5	1.5	2 x 25	100 µL
24 well	2 - 4 x 10^5	1	3	2 x 50	250 µL
6 well	10 - 15 x 10^5	3	9	2 x 100	1 mL

Table 3: Suggested transfection conditions for suspension cells

2. mRNA/RmesFect complexes preparation

a. *RmesFect*: Vortex the reagent and dilute the indicated quantity of RmesFect (refer to Table 3) in 25 to 100 µL of culture medium without serum and supplement.

b. *mRNA*: Dilute the indicated quantity of mRNA (see table 3) in 25 to 100 µL of culture medium without serum and supplement.

c. Mix up and down and incubate the mixture at room temperature for 15-20min.
Do not vortex or centrifuge!

3. Transfection

a. While the complexes are incubating, prepare your cells in serum-free medium (or serum-containing medium) and transfer the appropriate volume to the culture dish according to Table 3. In 24-well plate, for instance, plate 2x10^5 suspension cells just before transfection in 250 µL of serum free medium. Generally, serum-free condition leads to higher transfection efficiency.

b. Next, add the complexes directly onto the cells dropwise and all over the well. Important: gently mix complexes with the cells by pipetting the culture medium up and down (3-4 times) to disrupt potential cell clumps and to ensure contact of the complexes with the cells.

c. Incubate 3 to 6 h (4h is commonly used) in serum-free medium at 37°C under 5% CO_2.

d. If transfections are performed in serum free medium, add serum to adjust its concentration

e. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 6 to 24h following transfection.

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

IMPORTANT OBSERVATIONS

- Transfections are optimum when performed in the absence of serum. However, transfection can also be achieved directly in the presence of serum.
- WE suggest to promote as much as you can the contact between the cells and the transfection complexes. Consequently, here a few additional proposition:
  - Option 1, concentrate your cells: When the complexes are forming prepare your cells. Spin down the cells, resuspend them at 10×10 6 cells / mL in medium (serum free) and transfer the appropriate cell number to your well according to Table 2. Thereafter, mix the complexes with the cells, incubate 15 minutes and complete the culture medium as indicated in Table 2.
  - Option 2, promote contact by centrifugation: Centrifuge the plate after having mixed the cells with the transfection complexes for 2-3 minutes at around 1000-1200 rpm.

Protocol | Co-transfection

For co-transfection of several mRNA, mix the same amount of each mRNA and transfect as described above. For example, if you have two mRNA, mix 0.25 µg of each plasmid, complex the 0.5 µg of mRNA with 1.5 µL of RmesFect.

Optimization Protocol

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 4.
- Finally, culture medium compositions (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Tissue Culture Dish format	mRNA Quantity (µg)
96 well	0.125 to 1
24 well	0.250 to 2
6 well	1 to 8

Table 4: Suggested range of mRNA amounts for optimization (per well)

Additional products for your mRNA transfection experiments

- **RmesFect Stem** dedicated to mRNA transfection into stem cells
- **RmesFect CRISPR** dedicated to mRNA/gRNA transfection for your gene editing experiments

Purchaser Notification

Limited License

The purchase of this product 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). This reagent is intended for in-house research only by the buyer. Such use is limited to the purposes 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. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us. Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product 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). This document covers entirely the terms of the research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

This product 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.

www.bocascientific.com
(781) 686-1631
info@bocascientific.com