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RmesFect CRISPR transfection reagent

Protocol

For RNA (mRNA, gRNA) transfection in CRISPR/ CAS9 Genome Editing

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RmesFect CRISPR Reagent User Guide

Package contents	**RMC70500:** 500µl of RmesFect CRISPR reagent
Storage Conditions	Store at -20°C upon receipt
Product Description	RmesFect™ CRISPR transfection Reagent based on the TEE-technology is specifically designed for mRNA/gRNA transfection with high efficiency and low toxicity. RmesFect™ is efficient in a large variety of cells.
Important information	For Research use only. Not for use in diagnostic procedures

BEFORE YOU BEGIN:

• The RNA solution (mRNA, gRNA) and RmesFect CRISPR should be used at room temperature and be gently vortexed prior to use.

• All the complexes must be prepared in medium without serum and supplement.

• It is not recommended to use RPMI during complex preparation, prefer DMEM or PBS.

• For sensitive cells, medium can be replaced with fresh complete culture medium 4 to 6h after transfection.

Tissue Culture Dish	Cell Number per well	RNA (mRNA + gRNA) quantity	Total transfection volume per well
96-well	0.5 – 2.0 x 10^4	0.25 µg	0.2 mL
24-well	0.5 – 1.0 x 10^5	0.5 µg	0.5 mL
12-well	1.0 – 2.0 x 10^5	1.0 µg	1.0 mL
6-well	2.0 – 4.0 x 10^5	2.0 µg	2.0 mL
60 mm dish	0.5 – 1.0 x 10^6	4.0 µg	4.0 mL
90-100 mm dish	1.0 – 2.0 x 10^6	8.0 µg	8.0 mL
T75 flask	2.0 – 5.0 x 10^6	10.0 µg	12.0 mL

Table 1: Recommended cell number, total RNA quantity and transfection volume per well.

PROTOCOL STEPS

The following protocol is given for a single well of a 24-well tissue culture plate containing ~$1x10^5$ cells/well in 400 µL complete culture serum. If a different culture plate format is used, adjust cell number and reagent amounts according to the table 1.

NOTES: RmesFect CRISPR (RM) should be stored at -20°C. Use 3 µL of RM per µg total RNA.

1. Cas9 mRNA and gRNA solutions

A. Cas9 mRNA:
Dilute 0.5 µg mRNA into 50 µL DMEM without any supplement

B. Short guide RNA & Cas9 mRNA
Prepare a mix of gRNA and Cas9 mRNA in a 0.25:1 to 1:1 ratios (respectively gRNA to Cas9 mRNA) for a final quantity of 0.5 µg total in 50µL DMEM w/o supplement.

2. RmesFect solution

Dilute 1.5 µL RmesFect into 50 µL DMEM without any supplement.

3. Complexes preparation

Mix mRNA suspension or gRNA/mRNA solution with RmesFect CRISPR. Incubate the mixture for 20 min at room temperature.

4. Transfection

Add the complexes dropwise onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture. Incubate the cells under your standard culture conditions for 6 to 72h.

OPTIONAL:
Perform a medium change 2 to 6h after transfection. Withdraw the transfection medium and add fresh growth medium

Additional products for CRISPR Cas9 experiments:

- PolyMag CRISPR for Genome editing using expression plasmids
- ProDeliverIN CRISPR for Cas9 protein delivery
- ViroMag CRISPR to enhance transduction efficiency of CRISPR/Cas9 viruses

Purchaser Notification

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Product Use Limitations

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