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Stem cell: RmesFect Stem for mRNA transfection
± Lullaby Stem for gRNA transfection - For CRISPR/CAS9 – Genome Editing

The following protocol is given for a single well from 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 below:

Tissue Culture Dish	Cell Number per well	Total transfection volume per well
96-well	0.5 – 2.0 x 10^4	0.2 mL
24-well	0.5 – 1.0 x 10^5	0.5 mL
12-well	1.0 – 2.0 x 10^5	1.0 mL
6-well	2.0 – 4.0 x 10^5	2.0 mL
60 mm dish	0.5 – 1.0 x 10^6	4.0 mL
90-100 mm dish	1.0 – 2.0 x 10^6	8.0 mL
T75 flask	2.0 – 5.0 x 10^6	12.0 mL

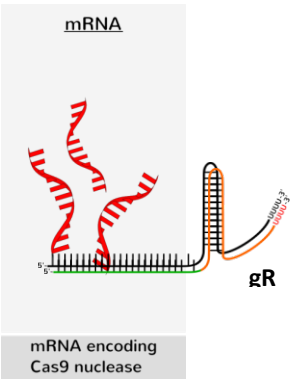
Table 1: recommended cell number and transfection volume.

Key parameter before beginning the procedure:

- The vector (DNA, gRNA, mRNA, protein, viral particles) and transfection reagents solutions should have an ambient 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.

NOTES: RmesFect (**RM**) and Lullaby (**LL**) should be stored respectively at -20°C and +4°C. Use **3 µL** of RM per µg mRNA and **4 µL** of LL per 50 to 125 nM gRNA.

1. OPTION 1: Cas9 mRNA and gRNA are delivered together



A. Cas9 mRNA: dilute **0.5 µg** mRNA into **25 µL** DMEM without any supplement

B. Short guide RNA: prepare a solution of gRNA for a final concentration of **50**-**125 nM** in **25 µL** DMEM without any supplement.

C. RmesFect solution: dilute **4 µL** RmesFect into **50 µL** DMEM without any supplement.

D. Complexes formation: Mix mRNA suspension with gRNA solution, gently pipette up and down several times and add the mix to RmesFect solution.

Incubate the mixture for **20 min** at room temperature.

2. OPTION 2: Cas9 mRNA and gRNA are delivered separately

A. Cas9 mRNA: dilute **0.5 µg** mRNA into **25 µL** DMEM without any supplement

B. Short guide RNA: prepare a solution of gRNA for a final concentration of **50**-**125 nM** in **25 µL** DMEM without any supplement.

C. RmesFect solution: dilute **1.5 µL** RmesFect into a new tube containing **25 µL** DMEM without supplement..

D. Lullaby solution: dilute **4 µL** Lullaby into a new tube containing **25 µL** DMEM without supplement.

E. Complexes formation

Add Cas9 mRNA suspension to RmesFect solution, incubate **20 min** at RT.

Add gRNA solution to Lullaby, incubate **20 min** at RT

3. 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 **24** to **72 h**.

Optional: perform a medium change 2 to 6 H after transfection. Withdraw the transfection medium and add fresh growth medium (optionally without antibiotics).