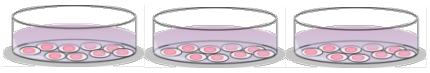
RmesFect™ Stem Quick Protocol

To find the ideal conditions, RmesFect™ Stem must be tested at ratios **2 µL/µg**, **3 µL/µg** and **4 µL/µg** (µL of RmesFect Stem / 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™ Stem (with 3 different amounts of reagent)

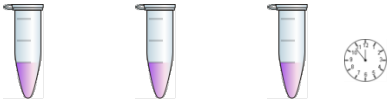
96 well plate	24 well plate	6 well plate
0.5µL/0.75µL/1µL in 25µL of serum-free medium or buffer*	1µL/1.5µL/2µL in 50µL of serum-free medium or buffer*	4µL/6µL/8µL in 100µL of serum-free medium or buffer*

4

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

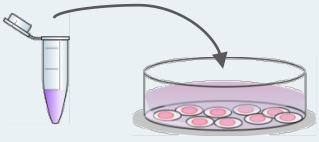
5

Incubate 20 min at room temperature



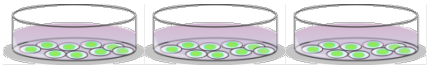
6

Distribute each mix dropwise onto the cells to insure uniform distribution



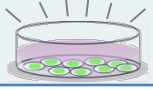
7

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



8

Choose the best ratio mRNA : RmesFect™ Stem



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***NOTES:**

(1) Of course the conditions provided above might required some further optimizations depending on your cells, RNA, readout etc…

(2) For cell lines, 24h before transfection seed the cells 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. For primary cells proceed as usual.
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).

(3) Allow reagents to reach RT and gently vortex them before forming complexes .

(4) **Medium or buffer without serum & supplement** must be used for the DNA/RmesFect Stem 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.

(5) For doses of RmesFect Stem less than 1µL, dilute the reagent with deionized water.

(6) For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.

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