

Reagents

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| cell types: | MEF |
| miRNA inhibitor: | miRCURY LNA™ microRNA Power Inhibitor 5 nmol, dilute 5 nmol in 250 µl H ₂ O = 20 µM stock solution, aliquot, store at -20°C |
| transfection reagent: | HiPerFect (Qiagen, Cat.no. 301705), 4°C |
| transfection medium: | serum-free MEF medium (DMEM + 1% Glu, + 1% P/S) or serum-reduced Opti-MEM I medium (Invitrogen Cat.no. 31985-062) |
| culture medium: | MEF medium (DMEM + 1% Glu + 1% P/S + 10% FCS) |
| protocol: | traditional protocol |
| material: | vortexer, round-bottom tubes (BD Falcon, Cat.no. 352063) |

Procedure

Day before transfection

- | 12-well | 6-well |
|--|---|
| <ul style="list-style-type: none">seed approx. 0.5×10^5 cells/in 1.5 ml medium per 6-well (test and adapt seeding density for individual MEF clones) | <ul style="list-style-type: none">1.0×10^5 cells/in 3 ml medium |

Day of transfection

- | 12-well | 6-well |
|---|---|
| <ul style="list-style-type: none">reduce medium to 1.1 ml | <ul style="list-style-type: none">2.3 ml (do not change the medium) |
| <ul style="list-style-type: none">dilute 20 µM stock solution 1:5 in sterile H₂O or serum-free medium to 4 µM working solution | |
| <ul style="list-style-type: none">for each transfection aliquot the following components into a round-bottom tube: | |

| | |
|--|---------------------------------------|
| 100 µl serum-free medium | 100 µl serum-free medium |
| 6-9 µl HiPerFect | 12-15 µl HiPerFect |
| 15-30 µl 4 µM miR-LNA inhibitor (50-100nM) | 6-12 µl 20 µM miR-LNA |
| inhibitor (50-100nM) | |
| $V_{\text{total}} = 1200 \mu\text{l}$ | $V_{\text{total}} = 2400 \mu\text{l}$ |

- vortex briefly and incubate 5-10 min at RT for complex formation
- drop-wise add 100 µl of transfection mixture onto cells per well, swirl gently
- incubate cell at 37°C/8%CO₂
- fluorescence can be monitored after 4-24h

1st Day after transfection

- change medium 24 h post-transfection, if required