

Transfection of pMiReport into Hek with transIT (Mirus)

B060

Jan Meier j.meier@dkfz.de

Michael Rogers

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1. plate $5-6 \times 10^4$ cells (per well) on a 24 well plate → final vol.: 500 μ l / well (make sure that cells are well distributed)
2. incubation 24h @ 37°C and 10% CO₂
3. add 1.5 μ l TransIT to 48.5 μ l medium, mix carefully and incubate @ RT for 10min (30 min at most)
4. dilute a total of 500ng plasmid into 150 μ l medium and add the TransIT mix (from step 3.)

Transfection of:

Renilla TK: 50ng

pMiReport: 10ng

control vector or miRNA vector: 440ng

final amount of transfected DNA: 500ng

5. incubate mixture @ RT for 30min
6. add 200 μ l of the plasmid TransIT mix to each well
7. incubate the cells for 24h
8. Harvest the transfected cells after 24h
9. wash the pellet with PBS
10. Lyse the cells with 65 μ l 1x "passive Lysis buffer" (Promega) , 15min @ RT, ~800rpm on a eppendorf shaker (freeze the lysate @ -20°C)
11. Measurement of Luciferase activity in triplicates (20 μ l of Lysate per well) using dual luciferase assay kit from Promega on a luminescence reader