

Chimera RNA transfection protocol

1. Seeding 293T cells at 10^5 cells/well (24 well plate).
2. Cells were washed with PBS once. Add 0.5 ml fresh DMEM without serum into cells.
3. Add 1 ul Lipofectamine 2000 into 30 ul DMEM without serum, mixing well and wait for 5 min at room temperature.
4. Add 1 ug of expression plasmid with 25 ul corresponding chimeraRNA (2 uM solution) in 30 ul DMEM without serum and mixing well.
5. Add Lipofectamine 2000 mixture from step 3 to the mixture from step 4. Mix well at room temperature for 20 min.
6. Add mixture from step 5 to cells at 24 well plate. Incubating at 37°C incubator for 4 hrs.
7. Exchange the medium with 0.5 ml fresh complete DMEM to each well of 24 well plate and incubate at 37°C incubator for overnight.
8. Exchange the medium with 0.5 ml fresh complete DMEM per well the next day and incubate at 37°C incubator for overnight.
9. Total cells were collected at 48 hrs after transfection for Western blotting.

Note:

This protocol is for overexpression and knock-down at the same time. If one wants to knock-down the endogenous one, it may take 72-96 hrs.

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