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Protocol for siRNA delivery using the targefect siRNA kit

(You can also use these protocols to deliver microRNA)

Set up cells to be transfected so that they are about 70% confluent at the time of the experiment.

Prepare transfection complexes as follows:

Use clear plastic tubes for complex formation. If kept frozen thaw the reagents and mix well before use (do not vortex)

Tube #	OptiMEM1	dsRNA	Targefect soln A	Targefect Soln B
1	1 ml	75 pmols	5 ul	15 ul
2	1 ml	75 pmols	5 ul	15 ul

Make aditions as follows: Add Optimem 1 first, then add dsRNA, mix well by flicking the tube about 12 times to create a vortexing action. Add targefect soln A next, mix well again by flicking the tube and then add targefect soln B, mix well again. Incubate the tubes at 37ºC for 25 minutes to form the transfection complexes.

Wash cells to be transfected twice with Opitmem 1. Aspirate the second wash completely. Add 1 ml of the transfection mix for 1 well for a 6-well dish (or for a 35 mm dish). Prepare 250 ul of transfection complex per well of a 12-well dish, 150 ul per well of a 24-well dish or 50 ul if using a 96-well plate Incubate the transfection complexes with the cells at 37^{o}C for 2 hrs. Add Complete media with serum (2 ml for a 35 mmm dish or one well of a 6-well plate, 1ml/well for a 12-well dish and 0.5 ml/well for a 24-well dish. Incubate overnight. Replace the media with fresh complete media the next morning and assy at 24-72 hrs post-transfection.

When using fluorescently labed dsRNA we have observed very efficient delivery into several cell types at 24 hrs post transfection.

For testing for silencing of transiently transfected genes we recommend transfecting the siRNA 12 hrs following transient transfection of the gene of interest and assaying for silencing of the transiently transfected gene 24 hrs after transfection of the siRNA.

For testing silencing of endogenous genes we recommend analysis 48-72 hrs after transfection of the siRNA.

Protocol for siRNA delivery using tragefect F-2 plus virofect

This protocol has been standardized in HUVECs but can be applied to other cell types or suspension cells. Data on siRNA delivery using Targefect F-2 plus Virofect has been kindly provided by Dr Michael Potente, Department of Cardioology, University of Frankfurt, Germany

1. HUVECs used were from from Cell Systems

2. The culture media is EBM with 10% FCS and supplements (Singlequotes)

3. HUVEC should be 80-85% confluent at the time of transfection

4. Be default we use 60 mm dishes for cell culture, therefore, the following protocol is designed for 60 mm wells

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Conditions:

- Add 500 ul DMEM (Optimem works as well) first
- Add 18 ul of the desired siRNA (20 uM) and mix
- Add 12 ul of F2 and mix
- Immediately add 25 ul of Virofect and mix
- Incubate for 20 min at 37°C
- In the meantime replace the culture media with 2 ml of fresh EBM 10% & suppl.
- Add siRNA complexes dropwise to the cells
- Change culture media the next day and assay for gene expression silencing after 24, 48 and 72 hours

Very recent data suggest that you can actually reduce the siRNA amount by half without losing any silencing effect. As you can see from the attached western blot, the silencing efficiency seems to greatly depends on the target sequence of the siRNA and not so much on the transfection method per se as all three methods gave comparable results. In our hands HUVECs work very well for the siRNA approach and we routinely get silencing efficiencies about 80 to 90% using different target genes. Nevertheless, the Virofect protocol is very easy to handle, suitable and time saving.

Protocol modifications for Co-delivery of DNA and siRNA:

Modifications suggested

To 800 ul of supernatant media covering cells in one well of a 6-well dish add 4 ul of Virofect. Incubate cells for 2 hrs. Then add 200 ul of the Tarnfection complex containing siRNA- Targefect F2 prepared as follows

Preparation of transfection complex
to 200 ul of DMEM add siRNA to a concnetration of 25 nM and 2 ul of Targefect F'2. Incubate 20-25 min at 37 o C . Add the transfection complex to cells pretreated with Virofect and incubate overnight.

If transfecting DNA plus siRNA then to 200 ul of DMEM add 1 ug DNA plus siRNA (add enough to give a 25 nM concentration) . Incubate at 37 o C for 25 min and add to cells pretreated with virofect as above.

We always recommend trying two different concentrations of siRNA - the concentration mentioned above and another which is 4 times higher.

Note: If using the Targefect siRNA kit to co-deliver DNA and siRNA we recommend using the same protocol above but omit the treatment with Virofect, and instead transfect using the TArgefect siRNA reagent complexed with the same ratio of DNA and siRAN as in the protocol above

Ie If transfecting DNA plus siRNA then to prepare transfection complex mix 200 ul of DMEM add 1 ug DNA plus siRNA (add enough to give a 25 nM concentration) with 2 ul of Targefect siRNA solution A and 4 ul of Targefect siRNA solution B and follow instructions as in the standard protocol for siRNA delivery using the Targefect siRNA kit.