Transfecting *Silencer*® Select siRNA into rat primary astrocytes

Invitrogen
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Read the complete protocol online:
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Lipofectamine® RNAiMAX Transfection Reagent is a proprietary, animal origin–free formulation for the transfection of siRNA into eukaryotic cells with low cytotoxicity. *Silencer*® Select siRNAs are high-performing siRNA molecules that incorporate the latest innovations in siRNA design, chemical modifications, and off-target effect prediction algorithms. This protocol provides a recommended procedure to transfect *Silencer*® Select siRNA Rat Primary Astrocytes using Lipofectamine® RNAiMAX Transfection Reagent.

Materials

Cell culture and transfection

1. **Rat primary cortical astrocytes** (Invitrogen, Carlsbad, CA, USA)
2. **Dulbecco's Modified Eagle Medium (DMEM) high glucose** (Invitrogen) **supplemented with 15% fetal bovine serum (FBS)** (Invitrogen)
3. **Opti-MEM® I Reduced-Serum Medium** (Invitrogen)
4. **PBS, pH 7.4** (Ambion, Austin, TX, USA)
5. **StemPro® Accutase® Cell Dissociation Reagent** (Invitrogen)
6. **Lipofectamine® RNAiMAX Transfection Agent** (Invitrogen)
7. **Silencer® Select siRNAs** (Ambion)

Methods: reverse transfection protocol

Prepare siRNAs

1. Resuspend the *Silencer*® Select siRNAs with nuclease-free water. A convenient stock concentration is 100 μM, which can be diluted to meet downstream experimental needs.
2. Validate the concentration of the siRNAs by measuring absorption at 260 nM using a spectrophotometer (e.g., Nanodrop) and adjust with water if necessary. Keep aliquots frozen at –20°C.
3. Dilute stock siRNAs of 100 μM to a working concentration of 10 μM. From the working stock, dilute siRNAs in 20 μL Opti-MEM® I per well to achieve a final concentration of 30 nM or your desired concentration (1–100 nM) in tubes or plates. Make master mixes when applicable for replicates to minimize variability (at least one well overage). For example, with a 10-μM siRNA stock, mix 0.33 μL siRNA (3.3 pmols) + 29.67 μL Opti-MEM® I per well or 1.3 μL siRNA + 118.7 μL Opti-MEM® I for the master mix.

Prepare cells

Prepare cells according to the manufacturer's cell protocol. Dilute cells to three different concentrations for the initial optimization experiment. For example, dilute primary rat astrocytes to 37,500 cells/mL, 75,000 cells/mL, and 150,000 cells/mL with cell growth media (in 50 mL polypropylene tubes). Place the tubes (with caps loosely tightened) in a 37°C incubator until ready for use.

Transfect cells in a sterile environment

1. Make three lipid dilutions (0.15 μL/well, 0.3 μL/well, and 0.6 μL/well) of Lipofectamine® RNAiMAX Transfection Reagent in Opti-MEM® I Reduced-Serum Medium for a total volume of 10 μL/well in a polystyrene 12 × 75 mm tube or conical tube. Make a master mix of sufficient volume to treat all wells to be transfected plus an extra 10% for pipetting variability. Mix by gently flicking the bottom of the tube.
2. Combine 10 μL Lipofectamine® RNAiMAX mixture per 20 μL diluted siRNA. Mix by tapping the tube or tap the plate back and forth to mix. Incubate this mixture for 20 min at room temperature.
3. After the incubation, add 80 μL diluted cells from the previous section to each well of a 96-well plate so that the final cell density per well is 3,000 cells/well, 6,000 cells/well, and 12,000 cells/well in a final volume of 110 μL.
4. Place the plate in a 37°C incubator under normal cell culture conditions. Media can be changed after 24 h. Remove and assay gene of interest expression levels at the desired time point (typically 24–48 h post-transfection).

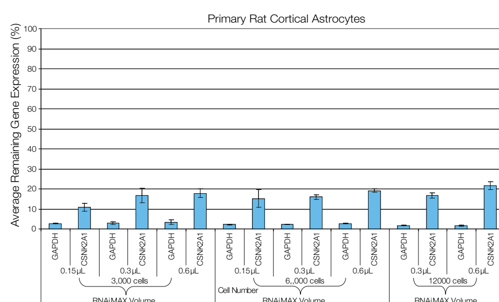

Figure 1. Robust knock-down of mRNA in primary rat cortical astrocytes transfected by Lipofectamine™ RNAiMAX Transfection reagent as measured by TaqMan® Gene Expression Assays.

Results

The primary rat cortical astrocytes were transfected with GAPDH and CSNK2A1 *Silencer*® Select siRNAs (30 nM) following the protocol described here. Analysis of mRNA knockdown was performed by qRT-PCR 24 h post-transfection. In order to achieve maximal knockdown of the target gene without causing cytotoxicity, the following conditions are typically varied and optimized: (*i*) volume of transfection reagent added per well, (*ii*) number of cells per well, and (*iii*) siRNA concentration. As can be seen from Figure 1, Lipofectamine® RNAiMAX reagent enables efficient siRNA delivery leading to strong knockdown at all three concentrations tested and at all three cell densities tested, without causing significant cellular toxicity. Lipofectamine® RNAiMax reagent works with a wide range of conditions with minimal to no optimization required, in contrast to other commercially available transfection reagents. GAPDH and CSNK2A1 *Silencer*® Select siRNAs at a low concentration of 30 nM induced >95% and >80% mRNA knockdown, respectively. The SVM algorithm enables selection of hyper-potent and highly specific siRNAs active at low concentrations that is beneficial in terms of minimizing off-target effects.

Contact

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