shRNA Transient Transfection Protocol

Step 1, Preparation of cells:

1. Approximately 18-24 hours prior to transfection, plate the appropriate cells (e.g. HEK293 for human, NIH3T3 for mouse or OLN-93 for rat shRNA validation) at 3 X 10^5 in 2 ml into the well of a 6-well plate. Grow the cells overnight in a 5% CO2 incubator to achieve 50% confluence.

Step 2, Preparation of the Turbofectin 8.0/DNA complexes (prepare immediately prior to transfection):

1. Add 50 uL of dH2O into each of the tubes containing shRNA expression plasmids. Vortex the tubes briefly to resuspend the DNA. The concentration of this solution is 100 ng/uL.

2. In a small sterile tube, combine the following reagents in the prescribed order. The order of reagent addition is important to achieve the optimal results.

    a. Dilute 1 µg of DNA in 250 uL of Opti-MEM I (Gibco 51985). Vortex gently.

    b. Add 3 uL of Turbofectin 8.0 to the diluted DNA (not the reverse order) and Pipette gently to mix completely.

    c. Incubate for 15 minutes at room temperature.

    cDNA expression plasmid for the target gene 0.01 ug to 1.0 ug (optional, available at OriGene)

Note: Add TurboFection 8.0 (or equivalent) directly into the serum-free media. DO NOT let the transfection reagent touch any plastic other than the pipette tip. For Dual-gene knockdown experiment, add 500ng of each shRNA expression plasmids (both pGFP-V-RS vector and pRFP-C-RS vector together) with 500 ng each of target cDNAs.

Step 3, Transfection:

1. Add the mixture prepared in Step 2 dropwise to the cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly.

2. Incubate the cells in a 5% CO2 incubator for 48 hrs before harvesting for RNA analysis and 72 hrs before harvesting for protein analysis.