siRNA Transfection of Mouse Bone Marrow-derived Macrophages

Ran Chen*

Department of Genetics, Stanford University, Stanford, USA
*For correspondence: rcchen@jfkbio.com

[Abstract] Short interfering RNAs (siRNA) are a type of double-stranded RNA molecule, typically 20-25 bp long, that are involved in the phenomenon of RNA interference. siRNA transfection is employed in this protocol to knockdown target gene expression in BMM'phi' cells. Two days after transfection with cells at 60-80% confluence, the knockdown efficiency can reach 90%.

Materials and Reagents

1. RPMI 1640 medium (RPMI) (Life Technologies, Invitrogen™, catalog number: 11875-093)
2. Fetal Bovine Serum (Atlanta Biologicals, catalog number: S10350)
3. Stock penicillin/streptomycin (P/S) (Life Technologies, Invitrogen™, catalog number: 15140-122)
4. Lipofectamine RNAiMAX (iMAX) (Life Technologies, Invitrogen™, catalog number: 13778150)

Equipments

1. Cell counter
2. 6-well plate
3. 24-well plate

Procedure

1. Isolation and culture of mouse bone marrow-derived macrophages (BMM'phi') (see Chen, 2011).
2. Use cell counter to count trypsinized BMM'phi's, and then split 2 x 10^6/ml cells into 6-well plates (for real-time PCR) or 24-well plates (for mycobacterial infection), in BMM'phi' growth medium overnight.
3. Mix-1: RPMI 500 µl (6-well plate) or 100 µl (24 well plate) 40 nM siRNA.

Mix-2: RPMI 500 µl (6-well plate) or 100 µl (24 well plate), 4.8 µl iMAx (6-well plate) or 1.2 µl iMAx (24-well plate). Add Mix-2 to Mix-1, briefly vortex and spin-down; incubate for 20 min at room temperature.

4. Change BMM'phi' culture medium to RMPI 2 ml (6-well plate) or 500 µl (24- well plate), add the transfection. Mix to the well and incubate at 37 °C for 4 h.
5. Change to BMM'phi' growth medium, incubate at 37 °C for 2 days. Now the cells are ready for further experiments.

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References

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