How to Produce and Use the siRNA Cocktails for RNAi Knockdown

QUAN KANG 11/3/2003; Edited by T.-C. He

dsRNA Transcription In Vitro Using T7 RiboMAX™ Express Large Scale RNA Production System

1. Prepare the linear DNA template.
2. Assemble transcription reaction at room temperature.

T7 Reaction Components	Sample Reaction
RiboMAXTM Express T7 2X Buffer	10.0µl
Linear DNA template(0.5-1µg total)	1-8µl
Nuclease-Free Water	0-7µl
Enzyme Mix, T7 Express	2.0µl
Total Volume	20µl

3. Mix gently and incubate at 37°C (water bath) for 1-2 hrs.
4. Incubate at 75°C for 5 minutes then leave the mixture on the hood to cool to room temperature.
5. Add RQ1 RNase-Free DNase to a concentration of 1unit(1unit=1µl) per microgram of template DNA to remove the DNA template and unincorporated rNTPs .
6. Incubate for 15 minutes at 37°C.
7. Extract dsRNA by PC-8.
8. Precipetate dsRNA by 100% ethanol and wash it by 70% ethanol twice.
9. Dissolve the dsRNA in nuclease-free water to a volume identical to that of the transcription reaction, store at -80°C.

dsRNA Digestion Using ShortCut RNase III (NEB) and siRNA Cocktail Purification

1. Transcript and purify dsRNA in vitro.
2. Assemble RNase III digestion reaction in a 1.5ml microfuge tube.

ddH_2O	75–x µl
10×ShortCut Reaction Buffer	10µl
dsRNA	x µl
ShortCut RNase(NEB)	5µl
10×$Mncl_2$	10µl
total volume	100µl

3. Mix and incubate at 37°C (water bath) for 20 minutes.
4. Add 10μl 10×EDTA to stop the reaction.
5. Remove the product to a Microcon G-30 tube, centrifuge at 14,000g for 15 minutes. Collect the siRNA in the tube, store at -80°C.

Tansfect siRNA Into Mammalia Cell Lines with Lipofectamine

For 24-well plate

1. Plate the cells in 24-well plate at 8-12hrs prior to transfection (approx 30-50% confluent).
2. prepare the Lipofectamine/siRNA mixture(Per well):
   100μl serum free DMEM + 3μl Lipo + 5~20μl siRNA
3. Mix well and incubate at room temperature for 20-30 minutes.
4. Meanwhile, remove the medium from every well, wash cell gently with 0.5-1ml of serum free DMEM.
5. Add 200μl serum free DMEM to each well, and return plate to incubator for 5-10 minutes.
6. Add the Lipo/siRNA mixture to each well, rock gently then return it to incubator.
7. After 3-5hrs, remove the medium and replace with 1.0ml complete medium.

Note: the amount of siRNA is up to your experimental purpose. For silencing the endogenous genes without any stimulation, try 5~10μl siRNA, but for silencing genes after stimulation(eg. After infection), try 10~20μl.