Protocol for Knockdown of HuR with siRNA

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[Abstract] Through a base-pairing-dependent mechanism, 21-23 nucleotide (nt) siRNA binds to complementary target mRNA to inhibit translation. In this protocol, knockdown of HuR with siRNA is described based on the mRNA degradation phenomena.

Materials and Reagents

1. HuR-siRNA (Life Technologies, Ambion®, catalog number: 4390824)
2. Control siRNA (Life Technologies, Ambion®, catalog number: Am4611)
3. OPITMEM (Life Technologies, Invitrogen™, catalog number: 3198)
4. Oligofectamine (Life Technologies, Invitrogen™, catalog number: 12252-011)
5. Culture medium
6. Dulbecco's modified eagle medium (DMEM)
7. Water (RNase free)

Equipment

1. 6-well plate
2. Flask T-75
3. Gloves

Procedure

A. For 6-well plate

1. Seed about 0.5-0.6 x 10^6 to 6-well plate (confluent is 1.2 x 10^6) ahead of one day. Culture media is DMEM for this cell line.
2. About 50% confluent. Ready for tranfection. The following steps are carried out at room temperature (RT).
3. For each well in 6-well plate, 20 µl 1 µM of SiRNA (stock 100 µM)or Ctrl SiRNA (stock 50 µM) mix with 175 µl of OPTIMEM, stand 5 min. mix 12 µl Oligofectamine with 48 µl of OPTIMEM, stand 5 min.

4. Mix the two solutions (255 µl) and let stand for 20 min.
5. Discard media from plate and wash once with OPTIMEM. Add 250 µl of OPTIMEM and the 255 µl mixture into well. Incubate 4-5 h. in 37 °C 5% CO_2. After the incubation, add 3 ml complete medium (10% FCS DMEM) and 600 µl extra FCS (become 15% FCS in the mixture media).

   This method usually is for pre-experiment. Once you are successful, you can use the flask method.

B. For flask T-75

1. Seed cells 2 x 10^6 (50% confluent) to T-75 flask ahead of one day (30 to 50% confluent is best).
2. 70 µl 1 µM of SiRNA or Ctrl SiRNA mix with 1,225 µl of OPTIMEM, 5 min –tube 1.
3. 84 µl Oligo with 336 µl of OPTIMEM, stand 5 min-tube 2.
4. Mix the two solutions (1,715 µl) and stand 20 min at RT.
5. Discard media from flask and wash one to twicq with OPTIMEM. Add 3.3 ml of OPTIMEM into well and mixture 1,715 µl, total 5 ml. Incubate 4-5 h. After that add 5 ml complete medium (10% FCS DMEM) and 1 ml FCS (final become 15% FCS in the mixture media). Confirm successful knockdown of HuR in pancreatic cell line by-PCR or by western blot.

Notes

All siRNA and control RNA should be kept on ice and the experimenter should wear gloves to take RNA tubes. You can knockdown other target mRNAs of proteins. This is just an example.