

RNA interference in THP-1 macrophages using nucleofection **Anne-Claude Gingras 2004/01**

This protocol is largely derived from the Amaxa guidelines (www.amaxa.com). I have obtained >95% knock-down in THP-1 cells using the protocol outlined below. RNA oligos are chemically synthesized and purchased through QIAGEN (www.qiagen.com). Other sources of RNAi (chemically or enzymatically synthesized) should also work, but I haven't tested this directly. This protocol also works for DNA transfection, although I always observe a significantly higher mortality with DNA transfections as compared to siRNA transfections. If using DNA, follow the same procedure (same program, same nucleofector solution, same quantity of cells), and use only 0.4 – 0.5 µg DNA. Before starting an experiment, you should also read the original protocols from Amaxa, as this protocol only provides conditions for the optimized protocol.

Required materials:

Amaxa nucleofector (www.amaxa.com)
Nucleofector kit V from Amaxa (www.amaxa.com)
THP-1 cells (www.atcc.org)
Chemically synthesized siRNA (www.qiagen.com)
Normal cell culture reagents
Reagents for the detection of the knock-down

Notes:

- 1) Cell growth: THP-1 from ATCC were maintained in RPMI + 10% FCS, and passaged when they reached a density of 7-8 x 10⁶ cells/ml. Cells were harvested for nucleofection when reaching a confluency of 3-5 x 10⁶ cells/ml. Cells were used at passages <15 after reception from the ATCC. The number of cells per transfection was 1.5 x 10⁶.
- 2) RNAi: The double-stranded RNA oligos were resuspended at 20nmol/ml, according to the manufacturer's instruction. Five (5) microliters of this suspension were used per nucleofection.
- 3) Nucleofection solution and nucleofector parameters: The nucleofection was performed using the nucleofector kit V. Several nucleofection programs were tested, initially on GFP DNA, and analyzed for viability and percentage GFP-positive cells. The most promising programs were then tested with siRNAs. Programs T-08 and T-12 performed the best under the conditions chosen.

Procedure:

- 1) Pre-warm RPMI + 10% FCS, and bring all nucleofector kit components to room temperature. For each nucleofection, you should prepare one sterile tube containing 500 μ l growth medium. Reconstitute the nucleofector solution according to the manufacturers' recommendations. Get the entire set-up ready, so that the transfection can be performed quickly (the nucleofector solution is toxic to the cells). The nucleofector apparatus can be installed in the hood for faster sample processing.
- 2) Harvest the THP-1 cells (1.5×10^6 cells/transfection $\times n$ transfections) via centrifugation (1000 rpm for 10 min). Remove medium, and resuspend in $n \times 100\mu$ l solution V.
- 3) For each nucleofection, use 100 μ l of this cell suspension and 5 μ l of the siRNA (see notes). Combine the cells and siRNA in a sterile eppendorf, and transfer immediately to a new electroporation cuvette.
- 4) Insert the cuvette in the nucleofector, select the appropriate program (T-08 or T-12 is a good start), and hit the start button. The pulse will be very short.
- 5) Immediately after the pulse, add some pre-warmed growth medium (from the eppendorf tube containing 500 μ l growth medium) to the cuvette (using the transfer pipette provided). Aspirate the cells from the cuvette, and transfer cells + medium to the eppendorf tube. Do not mix. Place immediately at 37 degrees.
- 6) Proceed in the same manner for the other samples.
- 7) Once all the transfections are performed, and the cells in the tubes have been incubating for about 10 minutes, transfer the entire contents of the tube to a multi-well plate (6 or 12 wells) containing an appropriate volume of medium.
- 8) Incubate for at least 24 hours. The knock-down was observed to last until at least 96 hours. Change the medium and expand the cells as needed.
- 9) Verify the extent of the knock-down using an antibody specific to the targeted protein. Alternatively, determine the extent of the knock-down at the RNA level, or with a co-transfected, epitope-tagged, target cDNA.