Cell Culture Transfection of HEK293 with cDNA and/or siRNA

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Abstract

Basic protocol to achieve lipofectamine transfection of Human Embryonic Kidney 293 (HEK293) cells with cDNA and/or siRNA.

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Guidelines

If protein yields are low following this method, it may be worthwhile to use complete DMEM without Pen/Strep. In this protocol, we op to use complete DMEM with Pen/Strep even though yields may be lower.

Before start

Make complete DMEM:

Reagent	***Volume***
DMEM	432.5 mL
FBS	50 mL
Pen/Strep	5 mL
HEPES (1M, pH 7.4)	12.5 mL

Materials

Opti-MEM™ Reduced Serum Medium 31985062 by Thermo Fisher Scientific
Lipofectamine™ 2000 Transfection Reagent 11668019 by Thermo Fisher Scientific
Human Embryonic Kidney (HEK293) Cells CRL-1573 by ATCC
Gibco Penicillin-Streptomycin (10,000 U/mL) (Pen/Strep) 15-140-122 by Fisher Scientific

Fetal Bovine Serum - Premium Select (FBS) S11550 by Atlanta Biologicals

HEPES BP310-500 by Fisher Scientific

HyClone Classical Liquid Media Dulbeccos Modified Eagles Medium (DMEM) SH3024301 by Fisher Scientific

Protocol

Prepare

Step 1.

One day prior to transfection, plate cells at a concentration of 500,000 cells per mL in a 12-well plate.

Prepare

Step 2.

Modify the transfection spreadsheet (attached) to calculate how much cDNA/siRNA is needed for experimental conditions. Make sure to include negative controls for the total amount of DNA or siRNA that is added.

Prepare

Step 3.

Sterilize the biosafety cabinet with 10% bleach for 20 minutes. Spray down the biosafety cabinet with 70% ethanol and use UV light for 15 minutes as a secondary decontaminant.

One Hour Prior to Transfection

Step 4.

Three wells at a time, aspirate all media from plated cells with a sterile glass pipette. Gently but quickly replace with 800 µL fresh warm complete DMEM, careful not to pipette vigorously enough to cause cells to detach.

NOTES

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Note: 1 hour must pass before transfection (step 10) but keep in mind there are 25 minutes total of waiting for solutions to settle in future steps.

Prepare Master Mixes

Step 5.

According to the transfection spreadsheet, label master mixes in 1.5 mL microcentrifuge tubes for each triplicate condition. First, add Opti-Mem to the master mix tubes.

NOTES

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Be as accurate as possible: for example, use P1000 to add 340µL, then P20 to add remainder e.g. 12.1 µL.

Prepare Master Mixes

Step 6.

Double check that concentrations of cDNA/siRNA match the transfection spreadsheet. Mix thoroughly by flicking, then add the cDNA/siRNA to the Opti-Mem master mix tubes.

Prepare Master Mixes

Step 7.

To the lipofectamine master mix: add lipofectamine to Opti-Mem, mix well, and then let the solution sit for 5 minutes.

Prepare Master Mixes

Step 8.

Add the diluted lipofectamine to cDNA/siRNA at 1:1 ratio. [+350 µL] Mix well. Let solution sit for at least 20 minutes.

AMOUNT

350 µl Additional info: 1:1 ratio to (cDNA/siRNA + Optimem) Master Mix

Transfect

Step 9.

On the plated cells, label wells in triplicate for each treatment. Be sure to number them to match the transfection spreadsheet. Mix the Lipofectamine + Master Mix solution thoroughly and add 200 µL of each respective mix in a careful, dropwise circular motion. Do not pipette directly on to cells in a stream.

AMOUNT

200 µl Additional info: from each respective Master Mix to plated cells.

Incubate

Step 10.

Mix the media with very gentle shaking, then return the cells to the incubator at 37°C and 5% CO_2.

Freshen Up the Media (Day 2)

Step 11.

The next day, aspirate the old media three wells at a time and add 1 mL of warm complete DMEM media.

Further Experiments (Day 3)

Step 12.

The following day, plan for cell lysis, protein assay, and gel sample preparation.

Warnings

- Human Embryonic Kidney (HEK293) cells are **biosafety level 2 (BSL-2)** and should be handled according to the CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines. They are considered BSL-2 not because they are inherently hazardous or infectious, but because of their potential to be infected with pathogens and in turn infect their handlers. Due to the impossibility to regularly screen this cell like for every human pathogen, **HEK293 cells should always be handled as potentially infectious**. Other BSL-2 cell lines include those positive for *Legionella pneumophila*, HIV, and other disease-causing pathogens in humans.

- Dispose of ALL waste that comes into contact with cells such as pipettes, gloves, and materials as biohazardous waste.

- Bleach all direct cell waste thoroughly. In our lab, our vacuum line tube empties in to a sealed waste jug with bleach already added to the bottom of it, making up at least 10% of the total volume. This way, aspirated media and cells immediately come into contact with the bleach. Before disposing of glass pipettes, we aspirate a small amount of 10% bleach through to clean both the pipette and tubing, then dispose of the pipettes as biohazardous sharps.