Jun 04, 2025

Transfecting COS-1 cells using FuGENE® 4K Transfection Reagent and cell lysis (M-PER) in a 6-well plate

DOI
dx.doi.org/10.17504/protocols.io.261ged1rov47/v1

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Protocol Citation: Jailyn Izu 2025. Transfecting COS-1 cells using FuGENE® 4K Transfection Reagent and cell lysis (M-PER) in a 6-well plate. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ged1rov47/v1

Protocol status: Working

Created: January 03, 2024

Last Modified: June 04, 2025

Protocol Integer ID: 92930

Abstract

General protocol for transfecting COS-1 cells with FuGene.

Materials

- 6-well plate (Corning, #3506)
- FuGene 4K Transfection Reagent (Promega, #E5911)
- Opti-MEM Reduced Serum Medium (Thermo Fisher Scientific, #31985062)
- Plasmid constructs
- Dulbecco's Modified Eagle Medium (DMEM) with GlutaMax (Thermo Fisher Scientific, #10569010)
- FBS (Gibco, #26140-079)
- Penicillin-streptomycin (Thermo Fisher Scientific, #15140122)
- PBS, pH 7.4 (Thermo Fisher Scientific, #10010023)
- M-PER mammalian protein extraction reagent (Thermo Fisher Scientific, #78501)
- Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, #78429)

Protocol materials

FuGENE® 4K Transfection Reagent **Promega Catalog #**E5911

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Seed cells

1 Cos1 cell line were purchased from (ATCC # CRL-1650) and cultured in DMEM with GlutaMax **DMEM with GlutaMax (Thermo Fisher Scientific, # 10569010)**supplemented with 10% FBS **Fetal Bovine Serum, certified # 16000044** and 1% **penicillin-streptomycin (Thermo Fisher Scientific, # 15140122)**.

2 Cells were maintained at 37 °C in 5% CO_2 and split 1:5 every 3-5 days based on the confluence (80-100 %) reached through visual monitoring. Cells were not passaged more than 30 times.

3 Seed COS-1 cells in a 6-well plate (*see specific protocols for seeding density and total volume amounts*). Incubate the plated cells in 37 °C with 5% CO2 (see specific protocols for duration).

Transfection

2d

4 Once cells have grown to 50-80% confluence, transfect. In a biosafety cabinet, combine FuGene and Opti-Mem (referred to in the Promega protocol as just "medium," volumes according to the manufacturer's protocol FuGENE® 4K Transfection Reagent **Promega Catalog #**E5911) in a 1.5 mL eppendorf tube. Vortex gently for 1-2 seconds and incubate at room temperature for 00:05:00 . 5m

5 Add plasmid constructs to each tube containing FuGene:Opti-MEM (ratio according to the manufacturer's protocol FuGENE® 4K Transfection Reagent **Promega Catalog #**E5911). Vortex briefly and incubate at room temperature for 00:15:00 . 15m

6 While incubating, the DNA with FuGene:Opti-MEM, aspirate the old media from the cells. Add 1 mL of fresh DMEM + 10% FBS + 1% pen-strep to each well.

7 After incubation, pipette 100 µL of the reaction mixture into the corresponding wells. Tilt the plate(s) back and forth to mix. 10m

8 Incubate the plate(s) in a 37 °C incubator with 5% CO_2 according to the manufacturer's protocol.

Lysis

10m

9 Aspirate the media from all wells.

10 Wash each well 3 times with 2 mL ice cold 1X PBS (filtered).

11 Add M-PER with 1X protease and phosphatase inhibitor to each well (*see specific protocols for volume)*

Be sure to keep M-PER and samples on ice.

Note: HALT protease and phosphatase inhibitor *must* be diluted in lysis buffer *immediately* before use (cannot be prepared the day prior).

12 Scrape the cells from each well 1 at a time using a cell scraper. Pipette all of the M-PER containing the cell lysate from each well into a clean 1.5 mL micro-centrifuge tube and immediately place the tubes on ice.

13 Centrifuge the tubes for 00:10:00 at 4 °C at 10000 rcf . 10m

14 Collect 100 µL aliquots of the supernatant and store them for future use (lysate that will be used within 1-2 weeks after harvesting can be stored at -20 °C , otherwise store lysates in -80 °C , per **Cell Signaling** recommendations).

Protocol references

Promega FuGENE® 4K Transfection Reagent (#E5911) Technical Manual:

https://www.promega.com/-/media/files/resources/protocols/technical-manuals/500/fugene-4k-transfection-reagent-protocol-tm694.pdf?rev=beb16dbcf2604534ace3cbe95b5080cb&sc_lang=en

HALT Protease and Phosphatase Inhibitor:
https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FMAN0011626_HaltProteasePhosphat_Inhibit_UG.pdf