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Version 1

COS-1 cell seeding, transfection, and lysis for protein expression assay V.1

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Jailyn Izu¹

¹NIMH



Christopher Bartley

Translational Immunopsychiatry Unit, NIMH

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Protocol status: Working

We use this protocol and it's working

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Abstract

General protocol for transfection and lysate preparation

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)



Transfect cells and lyse

3d

- 1 Follow the general protocol:

3d

Protocol

NAME

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

CREATED BY

Jailyn Izu

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Protocol-specific parameters:

- For seeding cells in **Step 1**, seed 500,000 COS-1 cells per well ( 1 mL total volume per well) and incubate the plated cells in  37 °C with 5% CO₂ for 24 hours  24:00:00 .
- For **Step 2**, transfect cells at 80% confluence and use a 1:3 DNA-to-FuGENE (µg/µL) ratio, with 2 µg of total DNA for each sample.
- For **Step 3**, transfect Flag wild-type, Flag-TBK1 N455S, or Flag-TBK1 M559R and co-transfect with EGFP (OmicsLink™ Expression Clone EX-EGFP-M11; GeneCopoeia, Inc., Rockville, MD)
- For **Step 6**, incubate transfected cells for 48 hours  48:00:00 , adding  1 mL of fresh media to each well after the first 24 hours.
- For **Step 9**, add  500 µL / well M-PER reagent + 1X protease and phosphatase inhibitor to each well