



Jun 04, 2025

Cycloheximide assay (Thermo Fisher Scientific, Catalog #AC357420010)

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v92mb4l3e/v1>

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Protocol Citation: Jailyn Izu 2025. Cycloheximide assay (Thermo Fisher Scientific, Catalog #AC357420010). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x54v92mb4l3e/v1>

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Protocol status: Working

We use this protocol and it's working

Created: April 17, 2024



Last Modified: March 26, 2026

Protocol Integer ID: 98346

Keywords: life of tbk1 n455, tbk1 n455, type tbk1, thermo fisher scientific, assay

Funders Acknowledgements:

National Institute of Mental Health

Grant ID: ZIA MH002982

National Institute of Mental Health

Grant ID: R01MH122471

National Institute of Neurological Disorders and Stroke

Grant ID: U01NS120836

Foundation for the National Institutes of Health

Grant ID: Deeda Blair Research Initiative for Disorders of the Brain

Abstract

Assay used to evaluate the half-life of TBK1 N455S as compared to wild-type TBK1.

Materials

- COS-1 cells
- EGFP plasmid (OmicsLink™ Expression Clone EX-EGFP-M11; GeneCopoeia, Inc., Rockville, MD)
- Flag-tagged TBK1 expression plasmid (OmicsLink™ Expression Clone EX-U0617-M11; GeneCopoeia, Inc., Rockville, MD)
- HA-tagged TBK1 expression plasmid (OmicsLink™ Expression Clone EX-U0617-M09; GeneCopoeia, Inc., Rockville, MD)
- Cycloheximide (Thermo Fisher Scientific, Catalog #AC357420010)
- Dimethyl Sulfoxide (DMSO) ()
- M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Catalog #78505)
- Halt Protease Inhibitor Cocktail, EDTA-free (100X) (Thermo Fisher Scientific, Catalog #78425)
- 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)



Seed cells

- 1 Seed 100,000 COS-1 cells per well ( 2 mL total volume per well) in a 6-well plate. Do not add CHX yet. Incubate the plated cells in  37 °C with 5% CO₂ for 48 hours.

Transfection

- 2 Follow Steps 2-6 in the general protocol for transfection:

1d

Protocol

NAME

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

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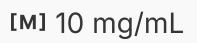
[Preview](#)

Protocol-specific parameters:

- For **Step 2**, use a 1:3 DNA-to-FuGENE (µg/µL) ratio, with 2 µg of total DNA for each sample.
- For **Step 3**, use the Flag-TBK1 plasmid constructs from "Flag-TBK1 N455S and Flag-TBK1 M559R cloning" protocol and co-transfect with EGFP (OmicsLink™ Expression Clone EX-EGFP-M11; GeneCopoeia, Inc., Rockville, MD)
- For **Step 6**, incubate transfected cells for 24 hours  24:00:00 .

Treat with cycloheximide

8h

- 3 Dilute cycloheximide (CHX) in DMSO to a final concentration of  10 mg/mL .
- 4 Add  20 µL of  10 mg/mL CHX (for a final concentration in the wells of  100 µg/ml CHX) or  20 µL DMSO (for controls) to the 24-hour time point wells only.

Note the time added.



- 5 After 08:00:00 add 20 μ L of [M] 10 mg/mL CHX (for a final concentration of [M] 100 μ g/ml) or 20 μ L of DMSO to the 16 hour time point wells only.

8h

Note the time added.

- 6 8 hours later, end the time course by follow Steps 7-12 in the general protocol for transfection:

Protocol

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

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Protocol-specific parameters:

- For **Step 9**, add 300 μ L / well M-PER reagent + 1X protease and phosphatase inhibitor to each well