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MG-132 (Invitrogen, #tlrl-mg132-2) and hydroxychloroquine (Sigma, #H0915-5MG) assay

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for evaluating the role for various protein degradation pathways on the half-life of TBK1 N455S

Materials

- COS-1 cells
- Cycloheximide (Thermo Fisher Scientific, Catalog #AC357420010)
- Dimethyl Sulfoxide (DMSO), filtered ()
- RNAse free H_2O, filter (Quality biological, #351-029-721)
- M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Catalog #78505)
- Halt Protease Inhibitor Cocktail, EDTA-free (100X) (Thermo Fisher Scientific, Catalog #78425)
- MG-132 (Invitrogen, #tlrl-mg132-2)
- Hydroxychloroquine (Sigma, #H0915-5MG)

Create stock solutions of MG-132 and HCQ

1 Obtain 5 mg MG-132 from Invitrogen (#tlrl-mg132-2)

2 Reconstitute MG-132 with 250 µL DMSO (filtered) to a final concentration of [M] 42 millimolar (mM) .

3 Create 25 10 µL aliquots and store in -20 °C . These are your stock solutions which are stable for up to 3 months. You will dilute these aliquots for the experiment.

4 Obtain 5 mg hydroxychloroquine sulfate (HCQ) from Sigma (#H0915-5MG)

5 Reconstitute HCQ with 250 µL RNAse free H_2O (filtered) to a final concentration of [M] 46 millimolar (mM) .

6 Create 25 10 µL aliquots and store in -20 °C . These are your stock solutions. You will dilute these aliquots for the experiment.

Prepare MG-132 and HCQ dilutions for the experiment

7 For MG-132, create a 1:21 dilution of 42 mM stock solution in media to obtain a [M] 2 millimolar (mM) dilution. You will add a volume of this to the cells at each time point.

*Perform dilutions needed for the experiment the **day of**, as dilutions of the stock solution should not be stored for more than 24 hours.*

8 For HCQ, create a 1:21 dilution of 46 mM stock solution in H2O to obtain a [M] 5 millimolar (mM) dilution. You will add a volume of this to the cells at each time point.

*Perform dilutions needed for the experiment the **day of**, as dilutions of the stock solution should not be stored for more than 24 hours.*

Perform cycloheximide assay

9 Follow the protocol for the cycloheximide assay, treating with CHX + MG-132 OR HCQ at time points (READ PROTOCOL-SPECIFIC PARAMETERS BELOW BEFORE BEGINNING).

Protocol

NAME

Cycloheximide assay (Thermo Fisher Scientific, Catalog #AC357420010)

CREATED BY

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Preview

Protocol-specific parameters:

There will be 3 conditions: (1) CHX alone, (2) CHX + MG132, and (3) CHX + HCQ). Therefore,

- For Step 4 you will,

(1) Add 20 µL of [M] 10 mg/mL CHX (for a final concentration in the wells of 100 ug/ml CHX) + 20 µL H_2O (filtered) to the 24-hour time point, CHX alone wells only.

(2) add 20 µL of [M] 10 mg/mL CHX + 20 µL of [M] 2 millimolar (mM) MG-132 to the 24-hour time point, CHX + MG-132 wells only.

(3) add 20 µL of [M] 10 mg/mL CHX + 20 µL of [M] 5 millimolar (mM) HCQ to the 24-hour time point, CHX + HCQ wells only.

- For Step 5 you will,

(1) Add 20 µL of [M] 10 mg/mL CHX (for a final concentration of [M] 10 ug/ml CHX) + 20 µL H2O to the 16-hour time point, CHX alone wells only.

(2) add 20 µL of [M] 10 mg/mL CHX + 20 µL of [M] 2 millimolar (mM) MG-132 to the 16-hour time point, CHX + MG-132 wells only.

(3) add 20 µL of [M] 10 mg/mL CHX + 20 µL of [M] 5 millimolar (mM) HCQ to the 16-hour time point, CHX + HCQ wells only.