VERSION 2

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

Inducing proteostasis stress using G-TPP V.2

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ABSTRACT

Inducing proteostasis stress in HeLa cells with G-TPP to analyse proteostasis protection and repair.

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Day 1

1 Seed cells in standard growth media, aiming for a density of ~80-90% the following day.

Day 2

2 Feed cells for 01:00:00 prior to starting treatment by aspirating the media from each well and replacing with a standard volume of growth media (eg. Feed with 2 mL of media if using a 6 well plate). 1h

3 Aspirate the media from each well/plate, and replace with standard growth media that has had G-TPP added at a final concentration of [M] 9 micromolar (µM) (for the DMSO control, add the same volume of DMSO as you added G-TPP to each well/plate).

Day 3

4 Harvest the G-TPP treated samples and DMSO control samples after the desired incubation period (eg. 2 – 12 h).

5 For samples to be analysed followed after G-TPP removal, aspirate the treatment media from each sample, wash each sample 3x in an ample amount of room temperature PBS (eg. 3 mL per well for a 6 well plate).

6 After 3x PBS washes, add an appropriate volume of standard growth media to each well/plate

(eg. 2 mL of standard growth media per well for a 6 well plate).

Day 4

7 Harvest the 24 h recovery (24 h R) time point, at 24:00:00 after the washout step (steps 5 – 6) was performed. 1d

8 Replace the media on the plate for the 48 hour recovery (48 h R) samples with an appropriate volume of standard growth media (eg. 2 mL of standard growth media per well for a 6 well plate).

Day 5

1d

9 Harvest the 48 h R samples, 24:00:00 after the samples had fresh standard growth media added (step 8). 1d