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Inducing proteostasis stress using G-TPP V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

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


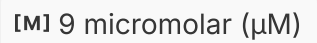
Troubleshooting



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  1M 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