



AUG 08, 2023

Inducing proteostasis stress

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ABSTRACT

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

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Protocol Citation: Louise Uoselis 2023. Inducing proteostasis stress.
protocols.io
<https://protocols.io/view/inducing-proteostasis-stress-cybdxsi6>

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

Created: Aug 08, 2023

Last Modified: Aug 08, 2023

PROTOCOL integer ID:
86085

Keywords: ASAPCRN

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 1 h 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).
- 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 9 μ M (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 R time point, at 24 h after the washout step (steps 5 – 6) was performed.

- 8 Replace the media on the plate for the 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

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