UPR Induction in Mammalian Cells

Hazards to be aware of:

- Sodium meta-arsenite, acutely hazardous, OSHA regulated carcinogen

Do not combine Sodium meta-arsenite and bleach, it forms extremely hazardous volatile arsenic oxides

- Tunicamycin, possible teratogen
- Thapsigargin, harmful irritant
- Dithiothreitiol (DTT)
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*** sodium meta-arsenite, tunicamycin, and thapsigargin are dissolved in DMSO, the use of nitrile gloves are strongly recommended when handling concentrated stock solutions.***

*****PLEASE REFER TO MSDS SHEETS AT END OF PROTOCOL FOR DIRECTIONS ON CLEAN UP IF A SPILL SHOULD OCCUR, AND APPROPRIATE FIRST AID MEASURES*****

PPE:

- Gloves
- Lab coat

Protocol

24 hours before induction, split cells to desired concentration.

1-2 hours before induction change the media (without antibiotics, or antifungal) allow to incubate 1-2hrs at 37°C. (10mL media for 10cm dishes)

Remove media into a conical, add drug, mix and replace media

DRUG:	**Final Conc:**	**Stock Solution:**
Sodium meta-Arsenite	10-100μM	100mM in DMSO
Tunicamycin	10μg/mL	10mg/mL in DMSO
Thapsigargin	200nM	2mM in DMSO
DTT	2mM	1M in H_2O

Incubate for various amounts of time, in incubator.

For timepoints take plates to your bench and work on top of bench coat. Aspiratior bottles should be fitted with a hepafilter between the bottle and the vacuum.

Remove drug containing media.

Arsenite should be discarded in arsenite waste container WITHOUT BLEACH. Tunicamycin, Thapsigargin, and DTT should be decontaminated in 10% bleach. Tm and DTT should be discarded in the appropriate waste containers. Tg can be poured down the sink after biological decontamination.

Wash cells 2X with PBS and collect cells into eppendorf with cell scraper. Spin down cells, aspirate PBS, and snap freeze in liquid N_2.

Clean UP:

Tissue culture plates should be disposed of in biohazard container. Cell scrapers can be sterilized in 70% ethanol and reused, or thrown into biohazard sharps container.