

## UPR Induction in Mammalian Cells

24 hours before induction, split cells to desired concentration in 10cm plates.  
CHO:  $2-3 \times 10^6$       3T3:  $1-2 \times 10^6$       HeLa:  $2-3 \times 10^6$

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 approx 7mL media into a conical, add drug, mix and replace media.

<b>DRUG:</b>	<b>Final Conc:</b>	<b>Stock Solution:</b>
Sodium meta-Arsenite	10-100 $\mu$ M	100mM in DMSO
Tunicamycin	10 $\mu$ g/mL	10mg/mL in DMSO
Thapsigargin	200nM	2mM in DMSO
DTT	1-2mM	1M in H <sub>2</sub> O

Incubate for various amounts of time, in incubator.

For timepoints, you can collect cells at the bench, unless using multiwell plates.  
In that case collect timepoints in tissue culture hood.

Cell collection:

Aspirate off media.

Wash cells 2X with PBS and collect cells into eppendorf with cell scraper. Spin down cells @ top speed 0.5m, aspirate PBS, and snap freeze in liquid N<sub>2</sub>.

**Clean UP:**

Aspirate media in container connected with hepa-filter and 10% bleach final volume. Tissue culture plates should be disposed of in biohazard container. Cell scrapers can be sterilized in 70% ethanol and reused.