**Trypan Blue Exclusion**

* 0.4% stock so dilute 1:10 to 0.04% (Can go down to 0.02% to be careful).
* Keep experiment the same as before (1mM, 16h exposure and 5x105 cell/ml).
* Do it in test tubes. Add the stain at the beginning yeast grow in it fine.
* Red channel on the microscope.
* Calculate proportion of dead/total.
* Have control (pdr3/1 + dye) and treatment (pdr3/1 + dye + insecticide).
* 8 replicates.
* At first try one with the above methodology. If there aren’t enough cells to visualise, increase the concentration at the beginning.
* Issue – the volume of insecticide stock required.
* do it in 1ml lots. 4µl stock per tube.

1ml of 1mM in culture tube:

**Test**

Make 0.6ml of 2mM solution from 500mM stock: 2.4µl stock + 597.6µl media.

0.4ml of 1.25x106 cell/ml yeast stock + 0.5ml of 2mM solution + 0.1ml 0.4% trypan blue stock

**Control**

0.4ml of 1.25x106 cell/ml yeast stock + 0.5ml media + 0.1ml 0.4% trypan blue stock