Insecticide Assay

* Final Well Volume = 200µl
* Plate reader settings: 30°C Number of cycles = 193, number of flashes per well and cycle = 22, cycle time = 300s, shaking options = double orbital, 200rpm, 272s, before each cycle.
* Create **4ml** 0.05 OD yeast culture stock and dilute down to final theoretical 0.01 OD.
* 1% final concentration for both solvents.
* P200 and P20 pipette.
* Fill plate perimeter with blanks to combat edge effect.
* Split plot randomisation with insecticides rotating clockwise each replicate.
* Repeat 8 times.
* 10 insecticides with 5 concentration values = 50 wells + 5 controls + 5 blanks = 60 wells.
* Make stock solutions **first.**
* Add yeast culture stock to all wells **last.**
* Do sulfoxaflor **last** as slightly different.

For All Insecticides Apart from Sulfoxaflor

Stock Solutions

1. **Make 200µl of 10mM stock solution** from 0.5M stock solution: 196µl media + 4µl 0.5 M stock solution.
2. **Make 200µl of 1mM stock solution** from 10mM stock solution: 180µl media + 20µl 10mM stock solution.
3. **Make 200µl of 0.01mM (10µM) stock solution** from 1mM stock solution: 198µl media + 2µl 1mM stock solution.

Well Construction

1. Using 10mM stock solution create **200µl** **5mM final well concentration**: 100µl 10mM stock solution + 58µl media + 2µl other solvent + 40µl 0.05OD yeast culture stock.
2. Using 10mM stock solution create **200µl 1mM final well concentration**: 136.4µl media + 20µl 10mM stock solution + 2µl other solvent + 1.6µl insecticide solvent + 40µl 0.05OD yeast culture stock.
3. Using 1mM stock solution create 800µl of **0.125mM stock solution** (with 1.25% solvent concentrations): 680.2µl media + 100µl 1mM stock solution + 10µl other solvent + 9.8µl insecticide solvent.
   * 1. Take 160µl of **0.125mM stock solution** and add 40µl 0.05OD yeast culture stock to create **200µl 0.1mM (100µM) final well concentration**.
4. Using 1mM stock solution create **200µl 0.01mM (10µM) final well concentration**: 154µl media + 2µl 1mM stock + 2µl other solvent + 2µl insecticide solvent + 40µl 0.05OD yeast culture stock.
5. Using 10µM stock solution create **200µl 0.001µM (1µM) final well concentration**: 136µl media + 20µl of 10µM stock solution + 2µl other solvent +2µl insecticide solvent + 40µl 0.05OD yeast culture stock.
6. Control – 156µl media + 2µl DMSO + 2µl methanol + 40µl 0.05OD yeast culture stock.

For Sulfoxaflor

Stock Solutions

1. **Make 200µl of 10mM stock solution** from 0.25M stock solution: 192µl media + 8µl 0.25M stock solution.
2. **Make 200µl of 1mM stock solution** from 10mM stock solution: 180µl media + 20µl 10mM stock solution.
3. **Make 200µl of 0.01mM (10µM) stock solution** from 1mM stock solution: 198µl + media 2µl 1mM stock solution.

Well Construction

1. Using 10mM stock solution create **200µl 2.5mM final well concentration**: 108µl media + 50µl 10mM stock solution + 2µl DMSO + 40µl 0.05OD yeast culture stock.
2. Using 10mM stock solution create **200µl 1mM final well concentration**: 136.8µl media + 20µl 10mM stock solution + 2µl DMSO + 1.2µl MeOH + 40µl 0.05OD yeast culture stock.
3. Using 1mM stock solution create 800µl of **0.125mM stock solution** (with 1.25% solvent concentrations): 680.4µl media + 100µl 1mM stock solution + 10µl DMSO + 9.6µl MeOH.
   * 1. Take 160µl of **0.125mM stock solution** and add 40µl 0.05OD yeast culture stock to create **200µl 0.1mM (100µM) final well concentration**.
4. Using 1mM stock solution create **200µl 0.01mM (10µM) final well concentration**: 154µl media + 2µl 1mM stock + 2µl DMSO + 2µl MeOH + 40µl 0.05OD yeast culture stock.
5. Using 10µM stock solution create **200µl 0.001mM (1µM) final well concentration**: 136µl media + 20µl of 10µM stock solution + 2µl DMSO +2µl MeOH + 40µl 0.05OD yeast culture stock.
6. Control – 156µl media + 2µl DMSO + 2µl methanol + 40µl 0.05OD yeast culture stock.

FILL PERIMETER WELLS WITH MEDIA