

## Hydrolase Extracellular Enzyme Assay for Wood Samples (Black Plates)

### Before starting

1. Check to see if there is enough buffer and defrosted substrate.
  - a. Frozen substrates should be defrosted a day in advance and mixed as necessary.

### Assay Protocol

1. Set up:
  - a. Wipe down the bench with ethanol.
  - b. Label 7 15 mL Falcon tubes for substrates: AG, AP, BG, BX, CBH, LAP, NAG
  - c. Label 2 50 mL Falcon tubes for standards: AMC, MUB
  - d. Set out the corresponding reservoirs and lids.
  - e. Set out the number of black plates to be used (# samples + buffer) and lids
  - f. Obtain the substrates from the fridge (keep stocks inside the box at all times)
2. Dilutions:
  - a. One substrate at a time, add **2.6 mL** stock into reservoir slot 1 and **2.6 mL** into the 15 mL Falcon tube.
  - b. Add **2.6 mL** ultrapure water into the 15 mL Falcon tube and vortex to mix (make sure the vortex goes all the way down to the bottom).
  - c. Take **2.6 mL** from the tube and fill reservoir slot 2.
  - d. Repeat steps b&c to fill reservoir slots 3-8.
  - e. Dilute **5 mL** of standard into **15 mL** water, vortex to mix.
3. Plate substrates:
  - a. Pour diluted standards and ultrapure water into corresponding reservoirs.
  - b. Set the 1200 uL multichannel pipette to **multi, d, 125 uL**, and the number of plates you have.
  - c. Follow the plate layout to plate the substrates.
  - d. Cover the plates with black lids when finished plating.
4. Plate homogenate:
  - a. Pour maleate buffer into the buffer reservoir.
  - b. Set the 1200 uL multichannel pipette to **multi, d, 125 uL, 8x**.
  - c. Dispense buffer from bottom to top of the plate in the buffer plate.
  - d. Set the manual 300 uL multichannel pipette to **125 uL**. Cut off tips to prevent wood particles from getting stuck.
  - e. Dispense the homogenate, one row at a time, from bottom to top of the plate.
  - f. *\*Take note of the time each plate was finished. Plates are read after 4 hours.*
5. Reading plates:
  - a. Start up the plate reader 10 minutes before the first plate is to be read.
  - b. Add the number of plates to be read and change plate IDs accordingly.

*\*Clean reservoirs/plates, wipe down the bench, and refill pipette tips when you are finished!*