24-Well Plate Assays

The following method was developed using Well Plate 24 Well 10 ml PP Sterile Individually Wrapped Plates (Thomson Instrument Company Cat # 931565-G-1X) and Gas Permeable Adhesive Seals (Thermo Scientific Cat # AB-0718).

Plate Set-up

1. Gather supplies need to complete the task.
2. All steps involving contact with culture or opening plates must be done in a laminar flow hood or equivalent biosafety cabinet.
3. Transfer materials required to the flow hood / designated work area (See Laminar Flow Hood. Use SOP LC-01-001-018 for further details on appropriate use of the hood).
4. Set up your work area in such a way as to prevent the need to move hands or equipment above open plates or lids.
5. Label the plate appropriately with at least the date, initials, and an identifier. If not labeling samples/treatment names, then record these somewhere else.
6. Distribute 5 ml culture to be tested to the required number of wells with P-10ML, P-5mL, or serological pipet.
7. Add any additional treatments required to each well, pipetting solutions directly into the culture with a clean pipette tip for each transfer. If data or timepoint “0” is required, then it is advisable to take the sample prior to making additions or allow cultures to mix on shaker after additions and prior to sample collection.
8. Seal the plate in such a way that the sticky underside of the seal is not touched by hand or comes into contact with anything else other than the plate. Position the seal so that all wells are fully covered and that facilitates opening the plate for sample collection. This allows the seal to be replaced while minimizing the risk of cross-contamination. There are multiple ways to achieve this. One way is to remove the smaller piece of the backing first and then attach the seal to one edge of the plate such that it sticks to the side panel of the plate. The backing can then be removed, and the seal put I place at the same time by pushing from the opposite end of the seal and rolling the backing off as the seal is put in place. This prevents any contact between the user and the plate or the sticky backing.
9. Slide a stainless-steel frame over/around the plate(s) to minimize “edge effect” issues.
10. Place plate into appropriate growth conditions. For culture of SE00107 this is 275rpm with the raised shaker table in box 1. Appropriate conditions for experimental goals may need to be determined prior to initiation.
11. Stop shaker prior to taking plates on or off shaker tables.
12. Clean up your work area.
13. Note that while theoretically it is possible to fit samples from up to four full 24 well plates onto a single 96 well plate for photometric data collection, this will not allow for “blank” wells. Plan the layout of your samples/data collection accordingly.

Data Collection

1. Gather supplies need to complete the task.
2. All steps involving contact with culture or opening plates must be done in a laminar flow hood or equivalent biosafety cabinet.
3. Transfer materials required to the flow hood / designated work area (See Laminar Flow Hood Use SOP LC-01-001-018 for further details on appropriate use of the hood).
4. Set up your work area in such a way as to prevent the need to move hands or equipment above open plates or lids.
5. For most experiments, data should be collected at 24-hour intervals, +/- 1 hour.
6. Stop the shaker and remove the plate(s). Note that the steel frames do not always fit tightly enough to lift the plate safely simply by grasping the frame; always pick the plate up by sliding one hand beneath the plate.
7. Restart the shaker to allow other plates to continue to mix.
8. Carefully remove the steel frame and peel back the seal, taking care to expose all wells without completely removing the seal from the plate or touching any part of the underside. If anything comes into contact with underside of seal, remove it, dispose appropriately, and use a new seal after sample collection.
9. Mix samples by pipetting up and down.
10. Sample wells by pipetting 200 µl from each and transferring to a 96 well plate. A sample format for up to four 24 well plates is shown in the example below. Ideally, a 6-channel adjustable spacer pipet (e.g., Rainin Cat # LA6-1200XLS) will be utilized to facilitate the sampling process, adjusting tip spacing accordingly when transferring liquid from the 24 well plate into the 96 well plate.
11. If you wish to replace volume lost to evaporation, add back 330 microliters of ddH20 to each well to account for volume lost due to evaporation over the previous 24 hours.
12. Once samples have been collected, fold the seal back over the plate and ensure proper sealing between wells, again taking care to not make contact with the underside of the seal. If necessary, discard the seal and position a new seal as described above.
13. Replace the metal frame.
14. Return the plate to the shaker and resume shaking at 275 rpm. Turn off shaker before adding or removing plates to prevent overspill. This step should be done as soon as possible after samples are taken to prevent settling of samples in the wells.
15. Collect data as described in SOPs specific to your data collection needs.
16. Clean up your work area.
17. Data should be reported as soon as possible following the completion of the experiment. With the current drawbacks of the system, particularly around evaporation and seals/lids it is not advisable to continue culture for more than ~7 days.