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Technical Bulletin

Running Multiple Amylase Strips

Salimetrics Alpha-Amylase Kinetic Reaction Assay Kit, Item # 1-1902

The Salimetrics alpha-amylase assay takes some practice to get used to the timing of the assay. Salimetrics' advice is to start slow – one strip at a time with controls – then work up to more strips as you get more comfortable and confident with the assay.

There are several options for running more than one strip at a time in an alpha-amylase assay:

- Use a multichannel pipette capable of delivering 320µL of amylase substrate to one set of 8 wells; you would have to change tips for each strip.
- Use a multichannel pipette capable of delivering 320µL of substrate to multiple sets of 8 wells; you would still have to change tips each time you need to refill (if you do multiple sets of strips).
- Use a manual or automated 96 well pipettor capable of delivering 320µL of amylase substrate to up to 96 wells at one time.

In order to avoid contamination of the substrate, discard pipette tips after each use; it is very important not to return any remaining substrate in or on the tips to the substrate reservoir. If your substrate becomes contaminated your amylase assays will not work properly. (Your substrate may turn yellow and/or your optical density (OD) values will rise.) In order to avoid running out of amylase substrate, use the last dispense cycle on the multichannel pipette. (There should only be a small amount of substrate left in the pipettes after dispensing if you use the last dispense cycle when pipetting.) The number of strips you can do at one time depends on the types of pipettes that you have available, how quickly you can pipette the samples into the wells and how quickly you can change pipette tips. Remember, it is only 8µL of sample and it will evaporate quickly.

The most critical factor to keeping the assay accurate and consistent is the 2 minutes between the readings. If the initial read (1 minute read) is at 50 seconds or 1 minute and 30 seconds, this will not invalidate the assay as long as the time between the two readings is exactly 2 minutes. Salimetrics recommends including controls on the first strip in the assay and the last strip in the assay. If the controls are in range, then your assay should be valid for all the strips in that particular assay.

Just to clarify, it is not necessary to change tips for each row/strip of wells on the plate when using the multichannel pipettors. (You can do 3 rows/strips with one filling of a 1200µL tip.) In order to avoid carryover, be sure not to touch the liquid in the wells to the tips when pipetting each row – this is difficult and takes practice. Change the pipette tips before re-entering the substrate trough. Salimetrics uses Biohit Proline or eLINE Electronic Pipettors (50 - 1200µL). Similar pipettors are available from several other manufacturers.

If bubbles are seen in the wells, they must be broken before the plate is read in order to get reliable results. Bubbles can be broken by 'popping' them with any clean sharp object. When a bubble is read in a plate reader, your results will usually be way out of line and many times will be a negative number. If you can't break the bubble in a timely manner, that sample should be repeated on another strip. ***To reduce the number of bubbles, Salimetrics highly recommends use of the reverse pipetting mode for the amylase assay.***