OHAUS®

Preparing a Fluorescence Assay and Avoiding Error

Developed by OHAUS Corporation

Standard Preparation

1. Determine your assay type and both the excitation and emission wavelengths.
   This can be determined based on the sample being analyzed and desired results.
2. Choose the highest gain and sensitivity that will allow for a maximum linear range.
3. Pipette a uniform volume of each reagent into the selected wells of a 96 well plate.
   The addition of identical volumes is crucial as well-to-well variations can cause significant error.
   Be sure to use the minimum required volume of each reagent.
4. Prepare replicates of a standard curve to use as a reference and for concentration determination.
5. After the addition of all reagents, mix the wells thoroughly through small pipette bursts.
   Avoid forming bubbles as they can cause a light scattering effect when reading the plate.
   Bubbles can be popped with either a needle or small pipette tip.
6. Briefly centrifuge the plate through the use of the OHAUS 5718R Multi-Pro Refrigerated Centrifuge (30314815) with the Microplate Rotor Attachment (30314824).
7. To avoid error, segregate bright samples through the use of empty wells.
   Also, be aware of edge effect and leave the outer wells empty as a buffer from plate bleed over.
8. After enough time has passed for the assay to be completed, use a fluorescence plate reader to obtain the final results.
9. Once read, discard the plate as a photo bleaching of the sample can occur while exposed to fluorescent light.

OHAUS Products Used Within This Procedure

5718R Multi-Pro Refrigerated Centrifuge

Microplate Rotor Attachment