OHAUS®

Quantifying Cellular Protein with a Bradford Protein Assay

Developed by OHAUS Corporation

Utilizing the OHAUS Guardian 5000 Hotplate Stirrer to incubate a sample during Bradford Protein Assay

For labs working with proteins, it is imperative that that they are able to determine protein concentration within a given solution. One way to determine this is through the use of a Bradford assay. The assay involves Coomassie Brilliant Blue G-250 dye, which causes a color change when interacting with the sample under certain conditions. This color change can then be correlated with a numerical value through an absorbance reader. Bradford Assays are a common practice as they are both rapid and highly sensitive.

Preparation of Protein Solution

1. Create a cell suspension by homogenizing the tissue sample using the OHAUS HT Lysing Homogenizer.
2. Remove a 10ml sample from the well-mixed cell suspension, and centrifuge it at 8,000g for 15 minutes with the OHAUS Frontier 5000 Series Multi Pro. Discard supernatant.
3. Wash pellets with 0.9% m/v NaCl, spin again, and resuspend in 2ml 0.2 M NaOH in a 28mm vial.
4. Install the base plate and the 28mm vial block on the OHAUS Guardian 5000 Hotplate Stirrer.
5. Turn on the hotplate and set the device to 100 °C. The hot top light will illuminate when the device gets above 40 °C.
6. Incubate for 10 minutes at 100 °C and remove the vial from the hotplate.
7. After cooling, centrifuge at 8,000g for 15 minutes. Collect supernatant for Bradford assay.

Bradford Protein Assay

1. In 96 well plate, carefully pipette 100µL protein assay dye 1x in each well. Take caution to not expose the assay dye to a direct light source for prolonged periods of time.
2. Add 20µL sample, mix well by pipette, then seal the plate.
3. Incubate at room temperature ~5 min. There should be a noticeable color change of the solution as the reaction takes place.
4. Read the absorbance with a plate reader at 595nm.

OHAUS Products Used Within This Procedure

OHAUS HT Lysing Homogenizer

OHAUS Frontier 5000 Series Multi Pro

OHAUS Guardian 5000 Hotplate Stirrer