Bradford Assay Tips

Thermo Scientific NanoDrop Spectrophotometers be used with the Bradford Assay to measure protein. The following information may be useful:

Variation between different kits

When comparing Coomassie G-250 and the Coomassie Plus Protein Assay kit from Pierce, preliminary results show the Coomassie Plus Protein Assay produces better reproducibility amongst triplicate readings than the regular G-250 stain when reading the samples directly after the optimal incubation time.

Sample Volume (Pedestal Measurements)

It is recommended that 2 ul volumes be used for all protein samples when making pedestal measurements on a NanoDrop™ Spectrophotometer. Always use an 8-channel pipettor when loading multiple samples onto the NanoDrop 8000 to minimize evaporation due to delays in sample loading. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestals as delays can compromise accuracy.

Time

Follow the manufacturer's recommended times for reaction incubations. Allowing the reaction to incubate for a longer than suggested time frame increases the potential for interfering aggregates. Higher protein concentration may increase the possibility of dye or dye-protein aggregates contributing to interfering light scattering.

pH

There may be signal output differences due to the change in pH when using diluents such as dH_2O and a neutral pH PBS buffer. Be sure to use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.

Mixing

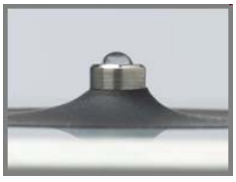
- When vortexing samples, especially when using small reaction volumes, use a mid-level rpm to avoid introducing micro bubbles into the fluid. Bubbles may negatively affect the readings.
- It is a good practice to lightly tap the vial to ensure homogeneity throughout the reaction volume before every reading. Micro-sampling non-homogenous solutions may produce fluctuation between replicate readings.
- Be cautious using centrifugation to clear aggregates as there may be residual protein loss as well.

Column Formation (Pedestal Measurements)

Repeated use of the Bradford reagent may change the surface properties of the pedestal resulting in samples "flattening out" rather than "beading up". Under such circumstances, the column may not form properly and result in erroneous measurements.

Use the instrument pedestal reconditioning compound (PR-1) as a rapid means of reconditioning the pedestals when the surface properties have been compromised and liquid columns break during measurement.

1. Open the vial containing PR-1 and use the applicator provided in the kit to remove a pin-head sized amount of the compound.
2. Apply a very thin, even layer of PR-1 to the surface of the upper and lower pedestals and let dry (30 secs).
3. Fold a clean, dry laboratory wipe into quarters and remove the PR-1 by aggressively rubbing the surface of the upper and lower pedestals until all compound residue is removed. The black appearance of the removed residue is normal.
4. Test the effectiveness of the re-conditioning by pipetting a 1ul sample of dH_2O (using a calibrated 2 ul pipettor) onto the lower measurement pedestal. The figures to the right show 1ul samples of dH_2O on properly conditioned NanoDrop Specrophotometer pedestals .



NanoDrop 2000/2000c

NanoDrop 8000

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Rev 2/09