**Protein quantification using the Amido Black Assay**

**(sample measured against a BSA standard curve)**

Schaffner & Weissmann , 1973. *Analytical Biochemistry*.

**A) Solutions to be made in preparation:**

1. **Washing solution**

* 10% acetic acid
* 90% MeOH (methanol)

1. Naphthol Blue Black ‘**Stain solution’**
   * 26mg of Naphthol powder
   * in 100ml washing solution
2. **Dissolving solution** 
   * 0.1M of NaOH solution (in dH2O)

**B) Method**

\* Do all washing and staining steps under fume hood as using acetic acid and methanol.

1. **Make stock** of 1mg/ml of BSA using the same liquid used to re-suspend sample protein pellet after extraction (ie urea buffer)
   * 10 mg BSA powder in 10ml urea buffer
   * vortex to ensure BSA is dissolved.
2. **Prepare serial dilution** of stock BSA in 1.5 ml tubes

|  |  |  |
| --- | --- | --- |
| **Final Dilution (mg/ml)** | **BSA stock needed (μl)** | **Urea Buffer needed (μl)** |
| 1 | 1000 | 0 |
| 0.8 | 800 | 200 |
| 0.6 | 600 | 400 |
| 0.4 | 400 | 600 |
| 0.2 | 200 | 800 |
| 0.1 | 100 | 900 |
| 0 | 0 | 1000 |

These can be stored in the fridge if in frequent use, or at -20 if infrequent. Don’t store for too long and for important samples, always make fresh standards.

1. **Adding Napthol blue black solution (***to stain the protein***)**

* Label **triplicate** 1.5 ml tubes for each BSA standard dilution and in them place:
* 50μl of the appropriate BSA standard
* 300μl of stain solution
* (ie you should have 21 tubes –> 3 for each of the 7 calibration levels)
* Incubate on the bench for 10 minutes
* Centrifuge at max (~17 ooog) rpm at room temp for 5 minutes

1. **Washing Pellet (***to remove any dye that is not associated with BSA***)**

* Pour off supernatant and drip on to tissue paper -> be very careful not to loose pellet!
* Watch pellet as you pour to take care it doesn’t get lost; re-centrifuge if necessary
* Add 500 μl of washing solution and vortex thoroughly.
* Centrifuge for 5 mins at 17 000g (room temp)
* Remove supernatant and wash as before

1. **Re-suspending Pellet**

* Dry tubes very well – leave in rack with lids open and put in the fume hood for a few minutes (but not too long as a ‘pruney’ pellet is very hard to re-suspend!)
* Add 1 ml of dissolving solution (0.1M NaOH) and vortex thoroughly to dissolve pellet.
* Leave on the bench for 10 minutes to ensure all protein is in solution

1. **Measure OD**

* Put spectrophotometer at 618nm
* Place 1 ml NaOH in cuvette and press blank. Keep this cuvette aside and blank between each sample (or every two samples if you have too many)
* Pour/pipette samples into cuvettes and take OD and record.
* This data will then be put in an excel sheet to create a standard curve.
  + A demo spreadsheet can be found on FEM lab github page -> wetLabSOPs -> proteinWork -> Amidoblack assay blank spreadsheet.xls
* Check R2 of standard curve and consider redoing if > 0.96

**Quantification of protein in samples:**

* Follow sample procedure for standard curve except use 50 μl sample instead of BSA
* Do on **same day & with same reagents as standard curve**
* To save sample (if vol is low) can do in duplicate instead of triplicate
* Dilute sample 1 in 10 or 1 in 5 if protein concentration is likely to be > 1mg/ml (ie outside range of standard curve) or if you want to save sample.
* In order to dilute do so **before** adding dye, ie take 5µl of sample and 45µl of urea, mix and then add 500µl of napthol Stain Solution. Then mulitply final [protein] by 10. In other words, if you discover at end of assay that your sample is out of range, do not dilute then and read again – result will be inaccurate. Start from beginning with sample diluted in appropriate buffer & do staining etc again.