PROTEIN EXTRACTION FROM CELL PELLETS AND QUANTIFICATION

Preparation of lysis buffer for protein extraction

Prepare 100 mL of Lysis Buffer:

- 50 mM Tris·HCl, pH 8.0
- 150 mM NaCl
- 1 mM EDTA
- 100 mM NaF
- 1 mM $MgCl_2$
- 10% Glycerol
- 1% Triton X-100
- bd-H_2O

Remember to add 1X Protease inhibitor cocktail (PIC) just before use (Stock 100X)

LYSIS BUFFER <u>100 mL</u>				
Reagent	**Molecular weight (g/mol)**	**Stock solution conc.**	**Final conc.**	**g or mL to add**
Tris·HCl, pH 8.0	\	1 M	50 mM	
NaCl	58.44	\	150 mM	
EDTA	\	0.5 M	1 mM	
NaF	41.99	\	100 mM	
$MgCl_2$	95.21	\	1 mM	
Glycerol	\	100%	10%	
Triton X-100	\	100%	1%	
bd-H_2O	\	\	\	

CELL LYSIS AND PROTEIN EXTRACTION PROTOCOL

- Add 500 µL of Lysis buffer to a 1.5 mL tube containing a thawed cell pellet (1 x 10^6 cells) and adjust with a proper volume of 1X broad-range protease inhibitor cocktail (PIC).
- Homogenize cell pellet using a plastic pestle until it is completely dissolved to promote cell lysis.
- Incubate sample on ice (+4 °C) for 15 min. Mix occasionally by vortexing.
- Centrifuge samples at 14,000 x g for 15 min, at +4 °C.
- Transfer the clear supernatant containing proteins into a new 1.5 mL tube.
- Supernatants can be used immediately for protein quantification, alternatively can be stored at -20 °C for long-term storage.

BRADFORD ASSAY FOR PROTEIN QUANTIFICATION

The Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 dye to proteins, primarily to basic (especially arginine) and aromatic amino acid residues (Bradford 1976). The dye exists in three forms: cationic (red), neutral (green), and anionic (blue) (Compton and Jones 1985). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (Amax = 470 nm). However, when the dye binds to protein, it is converted to a stable unprotonated blue form (Amax = 595 nm) (Reisner et al. 1975, Fazekes de St. Groth et al. 1963, Sedmack and Grossberg 1977). It is this blue protein-dye form that is detected at 595 nm in the assay using a spectrophotometer or microplate reader.

PROTEIN STANDARD FOR QUANTIFICATION: Bovine Serum Albumin (BSA)

Prepare a stock solution 2 mg/mL concentrated, then prepare 7 diluted aliquots of BSA at different concentrations (2, 1.5, 1, 0.75, 0.5, 0.25, 0.125 mg/ml) in 0.2 ml tubes:

Tube #	BSA volume (µL)	BSA source	Bd-H_2O volume (µL)	BSA final conc. (µg/mL)
1	20	2 mg/mL stock	0	2000
2	30	2 mg/mL stock	10	1500
3	20	2 mg/mL stock	20	1000
4	20	Tube 2	20	750
5	20	Tube 3	20	500
6	20	Tube 5	20	250
7	20	Tube 6	20	125
8 (blank)	/	/	20	0

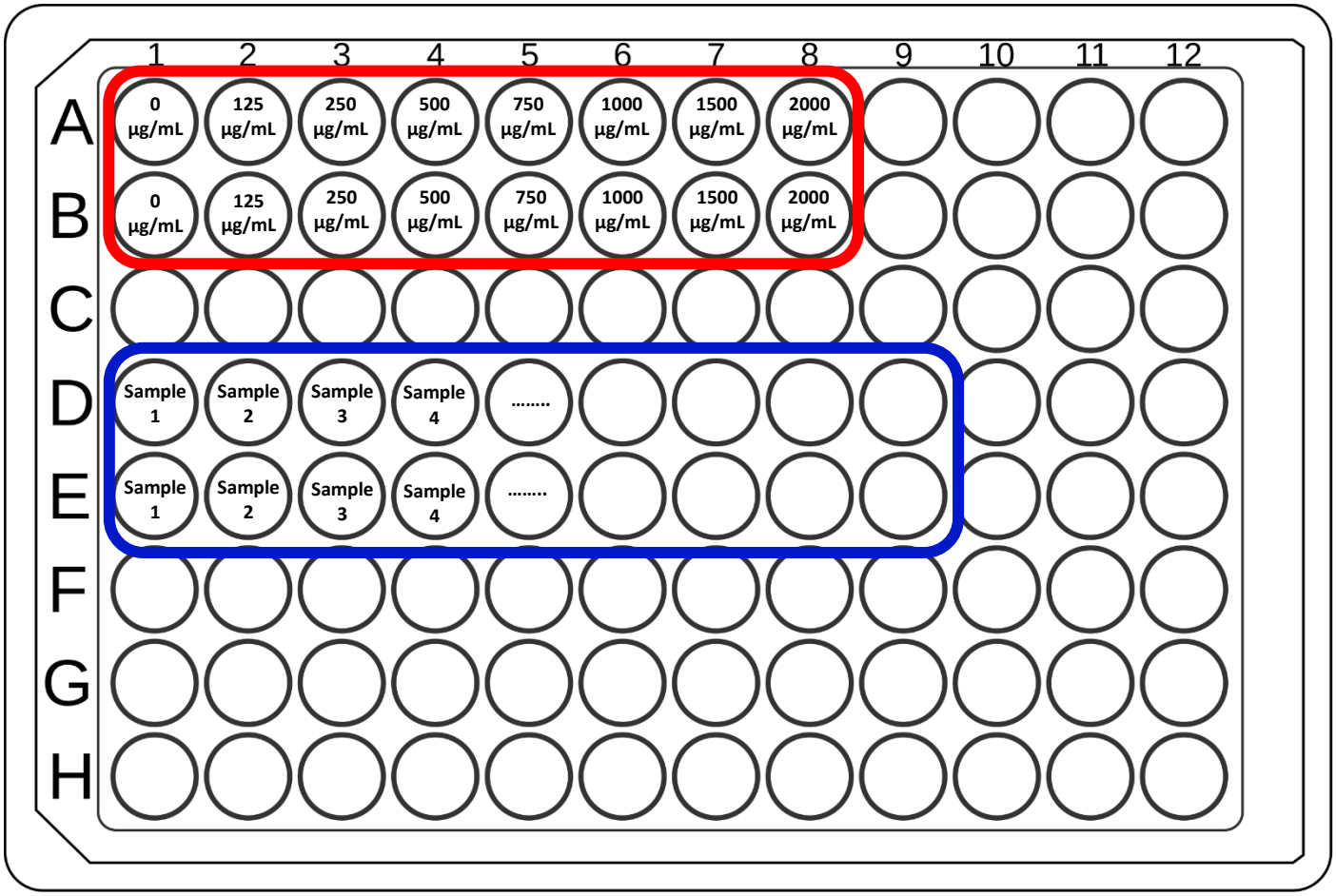
- Remove the 1x Bradford reagent from 4°C storage and let it warm to ambient temperature. Invert the 1x dye reagent a few times before use.

- Pipet 5 µL of BSA at different concentrations into a 96-well microplate. In the same way pipet 5 µL of unknown concentrated sample.

- Add 1x Bradford reagent to each well and mix carefully by pipetting. Replace with clean tips and add reagent to the next set of wells (Note: *if you dispense Bradford reagent starting from the well with the lower concentration of BSA to the one with the higher concentration you can use the same tip*).

Assay	Volume of BSA	Volume of 1X Bradford
Microplate	5 µL	250 µL

It is recommended to test each BSA dilution (standard point) and unknown sample in duplicate or triplicate:



- Incubate at room temperature for at least 5 min. Samples should not be incubated longer than 1 hr at room temperature.

- Set the spectrophotometer to 595 nm. Measure the absorbance of the standards and unknown samples.

DATA ANALYSIS

- Average the blank values and subtract the average blank value from the standard and unknown sample values.

- Create a standard curve by plotting the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis). Determine the unknown sample concentration using the standard curve. If the samples were diluted, adjust the final concentration of the unknown samples by multiplying by the dilution factor used.

  $Y = mX + q$ where $Y = Absorbance^{595}$; X= µg/ml protein

  $X = (Y - q)/ m$

  E.g. If the sample is 10-fold diluted → $X = [(Y - q)/ m] \times 10$

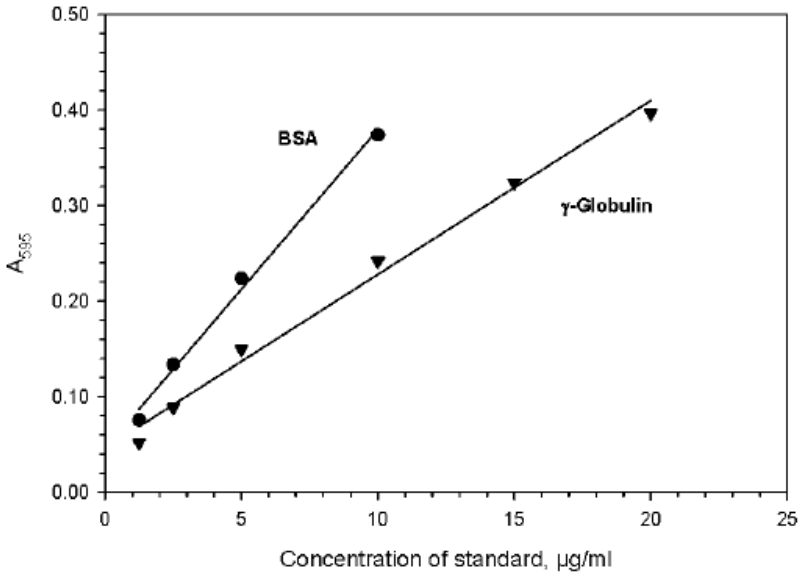


Fig 2. Typical standard curves using the microassay procedure with BSA and gamma-globulin standards.