Quantitative estimation of proteins by Bradford methods

Materials:

Chemical

Prepared crude extract, 40% pellet, dialyzed sample, BSA standard solution (2 g/l), Bradford reagent, distal water.

Preparation of solutions

1) Bradford reagent

- Dissolve 100 mg of Coomassie brilliant-G250 in 50 ml of 95% ethanol, add 100 ml of 85% w/v phosphoric acid and then complete the volume to 1 L by adding dis. H_2O.
- After the dye has completely dissolved, filter through Whatman#1 filter paper just before use.
- Filtration may have to be repeated to get rid of all blue components.
- Keep in dark bottle.

Equipment and Glassware

Micropipette, tips, plastic cuvettes, spectrophotometer.

Protocol:

1. In plastic cuvettes ad the following:

	BSA standard solution (5 g/l) volume (µl)	Distal water (µl)	Sample (µl)	Bradford reagent (µl)
Blank	**-**	**100**	**-**	**1000**
A	**5**	**95**	**-**	
B	**10**	**90**	**-**	
C	**20**	**80**	**-**	
D	**40**	**60**	**-**	
E	**60**	**40**	**-**	
F	**80**	**20**	**-**	
G	**100**	**-**	**-**	
Animal crude extract (D1)	**-**	**90**	**10**	
Animal crude extract (D2)	**-**	**80**	**20**	
Plant crude extract (D1)	**-**	**90**	**10**	
Plant crude extract (D2)	**-**	**80**	**20**	
40% pellet	**-**	**80**	**20**	
Dialyzed sample	**-**	**80**	**20**	

2. Mix the content of each tube.
3. Incubate for **15 min** at room temperature.
4. Read the absorbance at **595 nm** against blank.

5. Determine the protein contents from BSA standard curve.

Results:

Test tube	Protein concentration (µg/ml) [X- axis]	Absorbance at 595 nm [Y- axis]
Blank		
A		
B		
C		
D		
E		
F		
G		
Animal crude extract (D1)	_____	
Animal crude extract (D2)	_____	
Plant crude extract (D1)	_____	
Plant crude extract (D2)	_____	
40% pellet	_____	
Dialyzed sample	_____	