Protocol for Bradford Protein Determination Assay Using SpectraMax Plate Reader (M.Raizada)

Everything done at room temperature. Keep Bradford Stock Reagents on ice.
1. **For all pipetting less than 2ul, use P2 Pipettor.**

2. Prepare BSA Protein Standard Stocks in ddH20:

Stocks	
0.2ug/ul	use 1ul in 200ul Bradford=1ug/ml
1.0ug/ul	use 1ul in 200ul Bradford= 5ug/ml
	use 2ul in 200ul Bradord = 10ug/ml
	use 3ul in 200ul Bradord = 15ug/ml
	use 4ul in 200ul Bradford = 20ug/ml
	use 5ul in 200ul Bradord = 25ug/ml
	use 6ul in 200ul Bradford = 30ug/ml

Must do each of these in duplicate per 96well plate being read.

3. Prepare Bradford Reagent (kept as liquid 5X stock in 4C fridge; use as 1x)

Will need 200ul per sample.
Each sample in triplicate. Thus, 600ul per sample (plus 14 Standards plus 2 blanks):

To prepare 50ml 1x Bradford Reagent:
In a 50ml Orange-capped Falcon Tube:

Add ddH20	37.5ml
5xBradford Liq.	12.5ml

Vortex and invert, and keep on ice until use. The diluted 1x is stable at 4C for a few days.

4. Assign each sample a simple number. Photocopy and label a 96 well template as shown at the bottom. With all the standards and controls, you can do 24 samples in triplicate per plate.

5. Aliquot 200ul of 1xBradford per well using the multipi-pipettor. Pour the reagent into a square Petri Dish first to do this. NO BUBBLES !!!!!!!

6. Remove protein samples from freezer. Quick thaw in 37C bath, BUT DON'T leave there longer than necessary (1min should be enough). Invert each sample 3X to mix. If the sample has been completely assayed for Luc/GUS, then can keep at room temp.: otherwise, it MUST be placed on ice to prevent loss of protein activity.

7. Tape 96 well plate on top of labelled template.

8. Add BSA protein standards in the amounts listed at the top. Pipet up and down to mix, but avoid bubbles.

9. Add samples. Use 1.5ul unless Manish says otherwise. Pipet up and down to mix.

10. Let the prepared plate sit at room temperature for 10-15minutes.
11. Read plate and get a print-out.

12. If at least 2 of 3 readings per sample are not within 10% of each other, then the assay must be repeated again for that sample.