Consejo Superior de Investigaciones Científicas, Spain	EPP-SOP-CSIC01 Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Bradford Protein Assay

distribution list			
changes to prior version:			
	name	signature	date
experimenter 1	Huseyin Tas		16th April 2018

Instruction

Protein Running: Bradford Based Protein Concentration Calibration Method

1 Introduction / Purpose

Purpose of this protocol is to define how to prepare Bradford assay that is used for protein concentration assignments.

2 Equipment and chemicals

2.1 *Equipment*

Spectrophotometer

2.2 Chemicals

- Calibration Bradford - BioRAD Protein Assay Dye Reagent Concentrated (450 ml) Cat #: 500-0006
- Bovine Serum Abumin (BSA)

2.3 Bacterial strains

Tested and applied for *Pseudomonas putida* KT2440 derived proteins.

3 Media and buffers

Not applicable

4 Procedures

1. In Eppendorf tubes add 1000, 998, 996, 994, 992, 990 µL of ddH2O
2. Then, add 0, 2, 4, 6, 8, 10 µL of 1 mg/mL BSA solution sequentially toeach tube.
3. Separately, add 200 µL of the Bradford reagent dye into six spectrophotometer cuvettes
4. Take 800 µL of solution from each eppendorf and add to each cuvette.
5. Mix well by pipetting during this process
6. Incubate 10 mins at room temperature.
7. Measure the absorbance at 595 nm

5 remarks/troubleshooting:

Correlating the Calibration with the Protein Concentrations:

For a solution of unknown concentration measure the absorbance in a cuvette in the Spectrophotometer at 595 nm and compare the absorbance with that of BSA to find out the corresponding protein concentration in the solution.

6 Biosafety

No biosafety issues were associated with this protocol when applied to *Pseudomonas putida.*

7 Acknowledgements

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