Protocol 110_Detection of *E.coli* proteins via Western blotting

A. Reagents

1. Resolving gel buffer (Biorad cat no 1610798); 1.5M Tris-HCL (pH 8.8)

2. Stacking gel buffer (Biorad cat no 161799); 0.5M Tris-HCL (pH 6.8)

3. 10x Tris/Glycine/SDS buffer (Biorad cat no 1610732); Make 1 in 10 dilution with ddH_20 before use.

Final concentration for 1x buffer 25mM Tris, 192 mM Glycine and 0.1% (W/V) SDS at pH 8.3

4. Transfer 10x Tris/Glycine buffer (Biorad Cat no 1610734)

Make 1 in 10 dilution with ddH_20 before use

Final concentration for 1x buffer: 25mM Tris, 192mM Glycine and 20% (v/v) methanol at pH 8.3

5. Ponceau S

For 50ml: 0.05g Ponceau S, 2.5ml acetic acid, add ddH_2O to 50ml

6. Blocking agent

2%BSA+0.05% Tween 20 in PBS

7. Laemmli buffer (Biorad 1610737)

Add 50ul β-mercaptoethanol to 950µl laemmli buffer before use

8. Ammonium Persulfate (Biorad 1610700)

Dissolve 0.1g in 1 ml ddH_20 before use

9. TEMED (Biorad Cat no 1610801)

10.Washing buffer

0.05% Tween 20 in PBS

11. DAB color development solution

Dissolve 50 mg DAB in 100 ml TBS, add 10µl H_2O_2

B. SDS PAGE electrophoresis

1. Prepare Polyacrylamide gel (Please note that the percentage of the gel depends on the size of the protein to measured).

Resolving gel (10ml)	30% acrylamide/Bis	ddH2O	Resolving gel buffer	10% SDS	10% APS	TEMED
6%	2.0	5.3	2.5	0.1	0.1	0.008
8%	2.7	4.6	2.5	0.1	0.1	0.008
10%	3.3	4.0	2.5	0.1	0.1	0.008
12%	4.0	3.3	2.5	0.1	0.1	0.008
15%	5.0	2.3	2.5	0.1	0.1	0.008

Stacking gel (5ml)	30% acrylamide/Bis	ddH2O	Stacking gel buffer	10% SDS	10% APS	TEMED
4%	0.67	3.6	0.63	0.05	0.05	0.005

2. For 10ml of 10% Polyacrylamide gel, add 3.3ml of 30% acrylamide/bisacrylamide, 4 ml ddH_20, 2.5ml resolving gel buffer, 0.1ml 10% SDS, 0.1ml 10% APS and 0.008 ml TEMED.
3. Prepare 5ml of 4% stacking gel.
4. Add 0.67ml of 30% acrylamide/bisacrylamide, 3.6ml ddH_20, 0.63ml stacking gel buffer, 0.05ml 10% SDS, 0.05ml 10% APS and 0.005ml TEMED.
5. Add between 25 to 50µg protein to each well and run SDS-PAGE minigel for 1hr45 mins at 120V.

C. Western blot

1. Pre-wet PVDF membrane and extra think blotting paper before use in 1x transfer buffer.
2. Use semi-dry transmembrane for transferring the proteins onto the membrance and set up the gel according to the following order: Paper-gel-

membrane and paper).

3. Run the semi-dry transmembrane cell at 15V for 45 mins.
4. Stain with 1xPonceau S for 1-2 mins and destain with ddH_20.
5. Block membrane for 1-2 hrs at room temperature with 2%BSA+0.05% Tween 20.
6. Wash membrane and incubate with primary antibody for 1 hr on a shaker at 100 rpm.
7. The optimum antibody concentrations need to be determined prior to the experiment. Please read antibody optimisation section (section E).
8. Wash membrane 3 times for 5-10 mins in wash buffer on a shaker at 100 rpm.
9. Incubate with secondary antibody for 1 hr at room temperature.
10. Wash membrane 3 times for 5-10 mins in wash buffer on a shaker at 100 rpm.
11. Develop colour using DAP colour development solution.
12. Dry membrane overnight using paper towel and measure fluorescence to specific detection of protein.