



Sample Preparation

- 1 a) Add an equal volume of Sample Buffer (2x) to your sample.
or b) To prepare your own sample buffer (2x) use the following recipes:

Sample Buffer (2x) Catalogue Number	LongLifeGel BG – 165	Glycine BG – 145	Tris-Tricine BG – 125 [†]
SDS Electrophoresis Grade	0.4g	0.4g	0.8g
Tris	-	-	1.09g
Glacial Acetic acid	-	-	to pH 9.0
10x TBE Running Buffer	-	-	-
Glycerol	2.0mL	2.0mL	2.5mL
0.1% (w/v) Bromophenol Blue	1.0mL	1.0mL	-
0.1% (w/v) Phenol Red	-	-	0.2mL
0.1% (w/v) Coomassie Brilliant Blue	-	-	0.3mL
0.1% (w/v) Xylene Cyanole	-	-	-
0.5M Tris-HCl, pH 6.8	2.5mL	2.5mL	-
*2- β Mercaptoethanol	0.2 - 0.5mL	0.2 - 0.5mL	0.2 - 0.5mL
Deionised water to	10mL	10mL	10mL



Sample Preparation continued

- 1 Solubilising sample: Mix 100 μ l of sample buffer per mg of protein. Heat sample for 3-5 minutes at approximately 100°C. Clarify by centrifugation at 6000rpm for 3 minutes. Collect the supernatant.
- 2 If the sample is thermally labile, the SDS should be added to the sample as a 4% solution. The sample is left for 1 hour then an equal volume of solution containing 0.05M HEPES, 1M sodium chloride, 10% glycerol and 0.05% Bromophenol blue is added. Dissolution may be helped by sonication.

* If cleavage of disulfide bonds is required (reducing conditions).

† Add β -Mercaptoethanol for reducing conditions.

Sample Loading

As a guide, apply about 5-50 μ g (total protein) per sample well. Each sample well holds up to 50 μ l. For a sample with a total protein concentration of 10mg/ml, apply 2-5 μ L per well. Optimal sample size must be established by trials. Overloading will cause smearing and distortion. Excessive loading of proteins with free carbohydrate may also lead to band distortion or failure of the protein to penetrate into the gel. (See 'Trouble Shooting' page 19).