nUView Precast Gels

INSTRUCTIONS FOR USE

Running Conditions

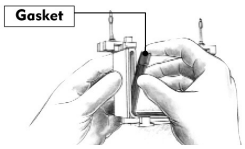
Connect the gel rig leads to the power supply and electrophorese according to below table.

		SDS Running Buffer				
		Tris-Glycine			Tris-HEPES	Tris-Tricine
Gel Selection		NG	NB	NN	NH	NH
Voltage		250	250	250	150	150
Approx. Current (mA/Gel)	Start	45	45	45	120	80
	Finish	15	15	15	50	30
Approx. Run Time per Gel (min)		30	35	55	45	45

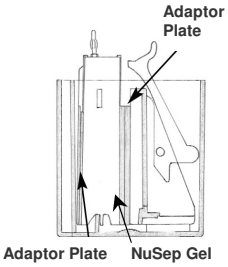
Remove the gel from its packet and place upright in the gel running apparatus. When running a gel, a tight seal must be formed between the gel cassette and the gasket of the running frame to prevent buffer and current leakage.

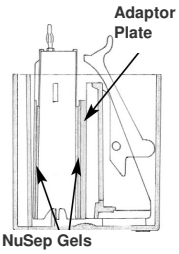
Note: nUView Universal Cassettes Only (NG)

Customers using a Bio-Rad® Mini-PROTEAN® must remove the gasket from the inner frame and turn the gasket around so the flat side is facing outwards and re-insert into the inner frame.



When using an Invitrogen™ running tank, adaptor plates should be used to assist in forming a tight seal. Invitrogen tank adaptor plate usage: Two adaptor plates are required when running just one gel (left) while one adaptor plate is required when running two gels (right).





Buffer Preparation

Pour sufficient running buffer into the inner tank of the gel running apparatus to cover the sample wells by 5-7mm. Fill the outer tank with running buffer to ensure proper cooling.

For best resolution, the buffer in the outer tank must reach the bottom of the sample wells to keep the gels cool. Using a transfer (pasteur) pipette, rinse sample wells thoroughly with running buffer to remove air bubbles and to displace any storage buffer.

Sample Preparation

Liquid samples: add an equal volume of TruSep Sample Buffer (2x) to your sample. Solid samples: solubilise the sample by mixing 100µL of 1X 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. If the sample is thermally labile, the sample is maintained for 1 hour at room temperature with intermittent mixing. Dissolution may be helped by sonication. If cleavage of disulfide bonds is required (reducing conditions) add 10 mM dithiothreitol or 5 mM β-mercaptoethanol final concentration.

Sample Loading

Apply about 5-50µg of protein per well. For a sample with a total protein concentration of 10mg/ml, apply 2-5µL per well. Use pipette tips specifically designed for gel loading.

Cautions: Use a pipette tip specifically designed for gel loading. Use of yellow tip for loading and inserting the tip too far into the cassette may cause the cassette to separate.

Note: Optimal sample size must be established empirically. Overloading may cause smearing and distortion or failure of the protein to penetrate into the gel.

Removing the Gel from the Cassette

At the completion of the electrophoresis run, remove the gel cassette(s) from the tank according to the manufacturer's instructions. To open the cassette, pry apart from one of the cassette edges (Figure 1). Pull the top plate of the cassette away from the bottom plate (Figure 2). The two halves will snap apart completely, exposing the gel. Loosen the gel at the bottom with water and remove the gel from the cassette.

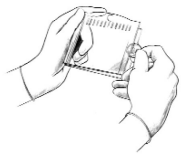


Figure 1

Figure 2

nUView Stain - 2 minute visualization

Briefly rinse the gel in RO water – **DO NOT SOAK.** Using UV safety glasses and gloves place the gel on a UV light box (250-300nm) and illuminate until bands are visible (approx 2 minutes).

UV bands will start to fade after approximately 7 minutes cumulative exposure to UV (stable if stored in the dark, though proteins may diffuse because they are not fixed). We recommend you capture (scan/photograph) the gel if you wish to keep a permanent record. The gel may be over-stained with any standard staining procedure.

All standard SDS staining procedures may be used with NuSep Gels.

Coomassie Staining

Immerse the gel in Coomassie Electrophoresis Stain (Cat # SG-021).

This stain can detect protein levels as low as 5 nanograms of BSA in a 1mm gel. Major bands are visible in 10 minutes. For maximum intensity, stain overnight. Destain in a 6% acetic acid solution.

Tip: Washing steps before staining will decrease the staining time, use 3 x 5 minutes in 200 ml water/gel.

Making Your Own Buffer (x10)

	Tris-Glycine (BG-143)	Tris-HEPES (BG-163)	Tris-Tricine (BG-121)
Tris	29 g	121 g	61 g
Glycine	144 g	-	-
HEPES	-	238 g	-
Tricine	-	-	90 g
SDS	10 g	10 g	5 g
Deionised Water	To 1000 mL	To 1000 mL	To 1000 mL

Further technical information is available on the NuSep web site, or call our technical support staff.

XNG2V.1

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