Gel Blotting

Follow the general guidelines for your blotting unit. RunBlue Blot Buffer (NXB82500) contains 0.25M Tris (base), 1.92M Glycine, and 1% SDS.

Dilute the Blot buffer:

- 10x for use in the RunBlue Dual Run & Blot System or semi-dry blotters (SDB)
- 20x for other Tank Blotters and for the XCell II™ Blot Module.

Equilibrate gels in 1x Blot buffer for 5 to 10 minutes prior to blotting. Equilibrate pre-cut Nitrocellulose (NC) or PVDF membranes in 1x Blot buffer for 3-5 minutes. (PVDF must be wetted in 100% methanol or ethanol prior to equilibration in buffer.)

Buffer Preparation	**RunBlue DRB**		**TB**		**SDB**		**XCell II™**	
	PVDF	NC	PVDF	NC	PVDF	NC	PVDF	NC
10x Blot Buffer (ml)	100		100		10		25	
Methanol (ml)	100	200	200	400	10	20	50	100
Ultrapure water (ml)	820	720	1740	1540	82	72	435	385

Blotting Conditions	**RunBlue DRB**	**TB**	**SDB**	**XCell II™**
Voltage (V)	200	50	25	35
Blot time (hours)	1 to 1.5	2 to 4	0.5 to 1	1 to 1.5
Expected current (mA)	180 (1 gel) 220 (2 gels)	250	250 – 300	200

Contact

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RunBlue™

Precast SDS-PAGE gel cassette

Instruction Manual

Introduction

RunBlue precast gels have superior rigidity and stability over traditional polyacrlyamide gels. For your convenience we have already removed the comb. The cassette locks the fingers in place and there is no tape to be removed.

Storage

Long term storage of up to 24 months store at 4°C or for 3 months at room temperature. For expiry date see box.

Sample Preparation

We recommend using RunBlue LDS Sample Buffer 4x which has been specifically formulated for use with our gels. The ions in the sample buffer match the gel buffer and it has a higher density, making it compatible with the density of the running buffer.

Reagent	**Reduced**	**Not Reduced**
Sample	**x** µl	**x** µl
Water	13 - **x** µl	15 - **x** µl
4x Sample Buffer (NXB31002)	5 µl	5 µl
10x Reducing Agent (NXA32001)	2 µl	--
Total volume	20 µl	20 µl

- Heat the samples, reduced or non-reduced, for 10 minutes at 70°C.
- Reduced samples should be run within 2 hours to prevent re-oxidation.
- Maximum volume that can be loaded in the wells is 35 µl.

Running Buffer Preparation

To enhance resolution our gels have been formulated with an improved ion system. **RunBlue SDS Running Buffer formulation must be used with these gels**.

Reagent	Volume
RunBlue 20X Run Buffer	40 ml
Ultrapure water	760 ml
Total volume	800 ml

Use RunBlue Rapid Run Buffer (NXB14500) for Reduced samples, and RunBlue SDS Run Buffer (NXB50500) for non-reduced samples. To run reduced samples in RunBlue SDS Run Buffer add 1 ml of RunBlue Antioxidant (NXA30010) to the formulation above. We recommend using fresh buffer for each run for both the inner and outer chamber. **Never use old buffers for the inner chamber (cathode).**

Sample Loading

Shortly before loading the samples, rinse the wells two times with ultrapure water. Use thin pipette tips to load samples near the bottom of the well.

Run Conditions

Place the RunBlue gel cassette in the tank so that the shorter plate faces the buffer core. When running one gel, use a buffer dam to seal the other side. Fill the inner (cathode) chamber with 200 ml fresh running buffer until it overflows into the outer (anode) chamber. Check whether the cell has been assembled properly so that there are no leaks, then pour at least 400 ml running buffer into the outer chamber. Run the gel(s) until the blue dye front nears the bottom of the cassette as follows:

Voltage	180 V
Start current	90 mA/gel
Ending current	40 mA/gel
Run time	30 - 70 min

Gel Staining

Remove the gel from the cassette into a staining tray and cover with 25 ml Instant Blue (ISB1L). Protein bands will be visible within minutes. Leave the gel in stain for at least one hour before transferring into water, if you wish to dry or store the gel. Alternatively store the gel in stain.

For silver staining, fix proteins for 10 minutes with a solution of 50% methanol, 10% acetic acid and 20mM sodium bisulfite. The sodium bisulfite can be added by diluting 1 ml of 800x Antioxidant (NXA30010) in 200 ml fixative. Substitute this fix step with the manufacturer's silver staining protocol and follow the remaining manufacturer's method.

Other gel stains can be used with RunBlue gels, please refer to protocols relevant to the specific stain.

Gel Drying

The gels can be dried without cracking between cellophane after equilibrating with RunBlue Gel Drying Solution (NXA04510).

1) Ensure that the gel has been staining for at least 1 hour. Further processing of the gel prior to completion of the staining process may result in protein destaining and reduced sensitivity. If this occurs simply restain the gel by incubating overnight in InstantBlue.

2) Submerse the gel in approximately 100 ml ultrapure water and incubate for at least 1 hour while gently rocking. Optionally adsorbent paper or paper towel can be added. Gels can be incubated overnight in water.

3) Incubate the gel in 'RunBlue gel drying solution' for 10 minutes and wet 2 cellophane membranes.

4) The gel is now ready for drying between the wetted cellophane membranes.