PROTOCOL

RunBlue™ Native Protein Gels

Applicable to: NXN20812, NXN20827, NXN32012, NXN32027
NXN01012, NXN01027, NXN02012, NXN02027
BCN20812, BCN20827, BCN32012, BCN32027
BCN01012, BCN01027, BCN02012, BCN02027

Release 1 © EXPEDEON 09/08/2018

INTRODUCTION

RunBlue™ precast gels have superior rigidity and stability over traditional polyacrylamide 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 at 4°C or for 3 months at room temperature. For expiry date see box.

SAMPLE PREPARATION

We recommend using RunBlue™ Native 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	VOLUME
Sample	x µl
Water	To 15 µl
4x Sample Buffer (NXB33010)	5 µl
Total volume	20 µl

- Do not heat the samples as that will result in protein denaturation
- 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™ Native Run Buffer must be used with these gels.**

REAGENT	REDUCED
20x RunBlue Native Run Buffer (NXB61500)	50 ml
Ultrapure water	950 ml
Total volume	1000 ml

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 up to 35 µl of each of the 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	42 mA/gel
Ending current	18 mA/gel
Run time	35 -90 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 in InstantBlue 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 at ~70°C (heat for 30s to 60s in a microwave oven). 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.

GEL BLOTTING

Follow the general guidelines for your blotting unit. RunBlue 10x Transfer Buffer (NXB82500) contains 0.25M Tris (base), 1.92M Glycine, and 1% SDS.

Dilute the transfer buffer:

- 10x for use in the RunBlue Dual Run & Blot System or semi-dry blotters (SDB)

- 20x for other Tank Blotters and (TB) and for the XCell II™ Blot Module.

Equilibrate gels in diluted transfer buffer for 5 to 10 minutes prior to transfer. Equilibrate pre-cut Nitrocellulose (NC) or PVDF membranes in 1x transfer 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 Transfer 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

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: www.expedeon.com/contact