PROTOCOL

RunBlue™ Bis-Tris Protein Gels

Applicable to: NBT00812, NBT00827, NBT01012, NBT01027, NBT01212, NBT01227, NBT41212, NBT41227
8% 10% 12% 4-12%

Release 2 © EXPEDEON 29/11/2018

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 or strip to be removed.

STORAGE

Long term storage of up to 12 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 (NXB32010) 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	NON-REDUCED
Sample	x µl	x µl
Deionized Water	6.5 - x µl	7.5 - x µl
RunBlue™ 4X LDS Sample Buffer (NXB32010)	2.5 µl	2.5 µl
RunBlue™ 10X Reducing Agent (NXA32001)	1 µl	--
Total Volume	10 µl	10 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 per well for 12 well gels is 35 µl. Maximum volume for 17 well gels is 25 µl.

RUNNING BUFFER PREPARATION

REAGENT	VOLUME
RunBlue™ 20X MES or MOPS running buffer	50 ml
Ultrapure water	950 ml
Total Volume	1000 ml

When running reduced samples, Expedeon recommends adding RunBlue™ Antioxidant to the 1X SDS Running buffer to provide the best possible resolution of bands.

Only use RunBlue™ Bis-Tris gels with MES (NXB70500) / MOPS (NXB75500) running buffers. Other Running Buffer formulas will not produce good results. Ensure buffers are correctly diluted with ultra-pure water if made from stock solutions. 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

NOTE: Please do not run NuPage® Bis-Tris gels and RunBlue™ Bis-Tris gels in the same tank at the same time, as it will adversely affect results for both gels.

MES BUFFER		MOPS BUFFER	
Voltage	200 V	Voltage	200 V
Start current	90-120 mA/gel	Start current	80-115 mA/gel
End current	40-60 mA/gel	End current	40-60 mA/gel
Run time	~ 45 min	Run time	~ 55 min

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 up to the top. Check whether the cell has been assembled properly so that there are no leaks, then pour approximately 600 ml running buffer into the outer chamber. Do not use less than 400 ml in the outer chamber. Run the gel(s) until the blue dye front nears the bottom of the cassette as follows:

GEL STAINING

Remove the gel from the cassette into a staining tray and cover with 25 ml InstantBlue™ (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. 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 gel drying solution.

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 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™ Tris Glycine SDS 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

*** You can now speed up your transfer to as little as 20 min using our new InstantBlot™ (NXB87500) transfer buffer.**

TECHNICAL SUPPORT

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