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ab139591 TEO-Tricine Precast Gels - RunBlue™ (8%, 12-well, 10x10cm)

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: NXG00812

View ab139591 TEO-Tricine Precast Gels - RunBlue™ (8%, 12-well, 10x10cm) datasheet:

www.abcam.com/ab139591

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This product is for research use only and is not intended for diagnostic use.

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1. Overview

TEO-Tricine Precast Gels - RunBlue™ 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.

2. Storage

Store at +4°C immediately on receipt. Alternatively, the gels can be stored for 3 months at room temperature.

3. Sample Preparation

We recommend using LDS Sample Buffer (4X) - RunBlue™ (TEO-Tricine) (ab119196) 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	(13 – x) µL	(15 – x) µL
LDS Sample Buffer (4X) - RunBlue™ (TEO-Tricine) (ab119196)	5 µL	5 µL
DTT Reducer (10X) - RunBlue™ (ab119199)	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.

Δ Note: *Maximum volume that can be loaded in the wells is 35 µL for 12 wells gels and 25 µL for 17 well gels.*

4. Running Buffer Preparation

When running reduced samples, We recommend adding Antioxidant (800X) - RunBlue™ (ab119200) to the 1X running buffer to provide the best possible reduction of bands. To enhance resolution our gels have been formulated with an improved ion system.

Run Buffer (20X) - RunBlue™ (TEO-Tricine) must be used with these gels (ab119197).

Reagent	Volume (mL)
Run Buffer (20X) - RunBlue™ (TEO-Tricine) (ab119197)	40
Ultrapure water	760
Total volume	800

Use Run Buffer (20X) - RunBlue™ (TEO-Tricine) (ab119197) for reduced or for non-reduced samples. We recommend using fresh buffer for each run for both the inner and outer chamber. Never use old buffers for the inner chamber (cathode).

5. Procedure

5.1 Sample Loading:

5.1.1 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.

5.2 Run Conditions:

5.2.1 Place the RunBlue™ gel cassette in the tank so that the shorter plate faces the buffer core.

5.2.2 When running one gel, use a buffer dam to seal the other side.

5.2.3 Fill the inner (cathode) chamber with 200 mL fresh running buffer up to the top.

5.2.4 Check whether the cell has been assembled properly so that there are no leaks, then pour approximately 400 mL running buffer into the outer chamber.

5.2.5 Do not use less than 400 mL in the outer chamber.

5.2.6 Run the gel(s) until the blue dye front nears the bottom of the cassette.

Voltage	180V
Start current	90 mA/gel
End current	40 mA/gel
Run time	30-70 mins

5.3 Gel staining:

5.3.1 Remove the gel from the cassette into a staining tray and cover with 25 mL InstantBlue™ (ab119211). 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.

5.3.2 For silver staining, fix proteins for 10 minutes with a solution of 50% methanol, 10% acetic acid and 20 mM sodium bisulfite. Substitute this fix step with the manufacturer's silver staining protocol and follow the remaining manufacturer's method.

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

5.4 Gel drying:

The gels can be dried without cracking between cellophane after equilibrating with gel drying solution.

5.4.1 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™.

5.4.2 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.

5.4.3 Incubate the gel in gel drying solution for 10 minutes and wet 2 cellophane membranes.

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

5.5 Gel blotting:

5.5.1 Follow the general guidelines for your blotting unit. Tris-Glycine Transfer Buffer (10X) - RunBlue™ (ab270518) contains 0.25 M Tris (base), 1.92 M Glycine, and 1% SDS.

Dilute the Transfer buffer:
1/10 for use in semi-dry blotters (SDB)
1/20 for use in other tank blotters (TB)

Buffer Preparation	TB		SDB	
	PVDF	NC	PVDF	NC
Tris-Glycine Transfer Buffer (10X) - RunBlue™ (mL)	100		10	
Methanol (mL)	200	400	10	20
Ultrapure water (mL)	1700	1500	80	70

5.5.2 Equilibrate gels in 1x Transfer buffer for 5 to 10 minutes prior to blotting.

5.5.3 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).

Blotting Conditions	TB	SDB
Voltage (V)	50	25
Transfer time (hours)	2 to 4	0.5 to 1
Expected current (mA)	250	250 to 300

*Δ **Note**: You can now speed up your transfer to as little as 20 minutes using Transfer Buffer - InstantBlot (ab270047) transfer buffer.*

Technical Support

For all technical or commercial enquiries please go to:

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