RunBlue™ TEO-Tricine SDS Protein Gels

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 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 (NXB31010) 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.

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>RunBlue™ 20X Run Buffer</td>
<td>40 ml</td>
</tr>
<tr>
<td>Ultrapure water</td>
<td>760 ml</td>
</tr>
<tr>
<td>Total volume</td>
<td>800 ml</td>
</tr>
</tbody>
</table>

Use RunBlue™ SDS Running Buffer (NXB850500) 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).

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 up to the top. 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: 180V
- 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 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 re-stain the gel by incubating overnight in InstantBlue™.

2. Submerge 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™ 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.)

<table>
<thead>
<tr>
<th>BUFFER PREPARATION</th>
<th>RUNBLUE™ DRB</th>
<th>TB</th>
<th>SDB</th>
<th>XCELL II™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (ml)</td>
<td>100</td>
<td>100</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Ultrapure water (ml)</td>
<td>820</td>
<td>720</td>
<td>1740</td>
<td>1540</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BLOTTING CONDITIONS</th>
<th>RUNBLUE™ DRB</th>
<th>TB</th>
<th>SDB</th>
<th>XCELL II™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage (V)</td>
<td>200</td>
<td>50</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>Blot time (hours)</td>
<td>1 to 1.5</td>
<td>2 to 4</td>
<td>0.5 to 1</td>
<td>1 to 1.5</td>
</tr>
<tr>
<td>Expected current (mA)</td>
<td>180 (1 gel)</td>
<td>250</td>
<td>250 - 300</td>
<td>200</td>
</tr>
</tbody>
</table>

1. BioRad Mini Protean Core
2. Remove the green gasket
3. Turn gasket 180° from original orientation
4. Guide gasket back into Core

*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