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Bjerrum Schafer-Nielsen buffer, modified by DING LAB, v1.0

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

This is a modified transfer buffer recipe for a semi-dry Western blot.

proportion guide

1

[M] 48 millimolar (mM) Tris Base

[M] 39 millimolar (mM) Glycine

[M] 15 % volume Isopropanol

pH 9.2

This recipe is modified from the original Towbin (1979) buffer, with increased Tris base but reduced glycine.

This recipe is suitable for semi-dry transfer, and ideal for SDS-PAGE and 2-D PAGE.

References:

Garfin DE and Bers G (1989). Basic aspects of protein blotting. In Protein Blotting: Methodology, Research and Diagnostic Applications, B.A. Baldo et al., eds. (Basel, Switzerland: Karger), pp. 5–41.

Towbin H et al. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76, 4350–4354.

2

for  1 L 10x stock buffer

add in a  2 L beaker

 58.2 g Tris Base

 29.3 g Glycine

add up to  800 mL ddH₂O , stir and mix well until all salts are fully dissolved

measure pH, and it should reach around  9.2

top up with ddH₂O to  1 L

3

before using, dilute the 10x stock buffer into 1x working buffer

for  1 L working solution

take  100 mL 10x stock buffer

add  150 mL Isopropanol

top up to  1 L with ddH₂O

Note

In the original recipe, the working buffer uses 20% ethanol, but here we changed it to 15% isopropanol.

4 **Tips (modified from Bio-Rad: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6211.pdf):**

1. Use high-quality, analytical grade reagents to enable better buffer conductivity and hence better transfer.
2. If reusing the buffer, measure the pH before use and make sure it maintains at  9.2 .
3. Do not further dilute the transfer buffer from 1x to the levels below the recommended concentration, because this decreases the buffering capacity of the buffer.
4. Do not adjust the pH of the buffer, because it can result in increased buffer conductivity, manifested by higher initial current output and decreased resistance.
5. There is no SDS in this recipe, but it can be added when it is necessary. Increasing SDS in the transfer buffer increases protein transfer from the gel but decreases the binding of the protein to the nitrocellulose membrane. In this case, nitrocellulose membrane can be substituted by PVDF membrane when SDS is used in the transfer buffer.
6. Addition of SDS increases the relative current, power, and heating during transfer, and may also affect the antigenicity of some proteins.
7. Increasing alcohol in the transfer buffer decreases protein transfer from the gel and increases the binding of the protein to the nitrocellulose membrane.