Dec 24, 2020

2. Gel run and transfer -Tricine

Elizabeth Fozo[1]
[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

Western Blot

PROTOCOL CITATION

Elizabeth Fozo 2020. 2. Gel run and transfer -Tricine. **protocols.io** https://protocols.io/view/2-gel-run-and-transfer-tricine-bqqtmvwn

KEYWORDS

Western Blot, Tricine, SDS running, Buffers

CREATED

Dec 14, 2020

LAST MODIFIED

Dec 24, 2020

PROTOCOL INTEGER ID

45555

GUIDELINES

Western bolt

- Buffers
- Running gel
- Protein Transfer
- mini bolt protocol

ABSTRACT

Western Blot

BEFORE STARTING

Prepare 1X Tris-Glycine Transfer Buffer using 10X as follows:

- Tris-Glycine Transfer Buffer 100 ml
- Methanol 200 ml
- Deionized water 700 ml

Buffers:

1 Tricine SDSRunningBuffer- dilute from 10 X to 1X

Composition 10X/L

- 100 mM Tris base (121 g)
- 100 mM Tricine (179 g)
- 0.1% SDS (10g)

pH 8.3

The pH of the 1X solution is 8.3. **Do not** use acid or base to adjust pH (Novex, 2003)

Stable 6 months at room temperature

2 Tris-GlycineTransferBuffer-(Novex, 2003)

Composition 10X/L

- 12 mM Tris Base (14.56gm)
- 96 mM Glycine (72 g)

pH 8.3(1X solution is pH 8.3, **do not** use acid or base to adjust)

Mix well and adjust the volume to 1 L. Store at room temperature, stable for 6 months.

Different concentrations found from different protocols e.g. this one was suggested for BioRad'selectrophoresis transfer cells

TowbinBuffer with SDS

25 mM Tris (3.03 gm/L),192 mM Glycine (14.4g/L), 20% MeOH (200 ml), SDS (0.1 g/L)- do not use SDS for small protein

Running gel (See Invitrogen quick reference Cat. No. A25977, publication part no. 100025990).

3 If running only one gel- make sure the unused chamber does not contain the cassette clamp.

3.1 Prepare buffer (dilute 10X Tricine SDS Running Buffer to 1X)

3.2 Plan to load 10 ul and with 5 ug of protein (might vary based on the concentration of your protein of interest). Prepare 1X sample buffer for dilution of samples, if needed. Allocate 12 ul (sample in SB containing 0.5 ug/ul) to an Eppendorf tube, also prepare your standard (1-1.5 ul of SeeBluePlus2 + 1X Sample buffer). Heat at 85 C for 2 minutes.

3.3 Prepare gels.

- Remove the comb, and rinse wells three times using 1X Running Buffer.
- Remove the white tape near the bottom of the gel cassettes and place the gels in the tank.
- Load 10ul of your sample and standard. Adjust gels as necessary.
- Fill up the tank with Running buffer and Run (125 V constant ~90 minutes).

Protein Transfer (see mini blot module Cat. No. B1000)

4 Cut selected transfer membrane and filter paper to the dimensions of the gel

4.1 PVDF membrane: Pre-wet PVDF membrane for 30 seconds in **methanol**, ethanol, or isopropanol.

Briefly rinse in deionized water, then place in a shallow dish with 50 ml of 1X Transfer Buffer for several minutes.
Standard PVDF has an unacceptable background signal in the IR. Handle the membranes with forceps at the very edges, and minimize contact with gloves. Damage to the membrane can show up on the image of the blot. **Use only dishes that are clean and have not contained Coomassie blue** – the instrument is very sensitive and will detect traces of blue dye on the blot. The blue dye in the pre-stained markers will be detected, which is convenient. The red marker in the set will not be detected.

4.2 Nitrocellulose: Place the membrane directly into a shallow dish containing 50 ml of 1X Transfer Buffer for several minutes.

4.3 Filter paper: Soak the filter paper briefly in 1X Transfer Buffer immediately prior to use.

4.4 Gel: Use the gel immediately following the run. Do not soak the gel in transfer buffer.

Follow **mini blot module protocol** (20V- 50 minutes for PVDF membrane)

5
- After the transfer is complete, turn the power supply off, unplug the power leads, and remove the lid
- Remove the blot module from the chamber and empty the transfer buffer, open module assembly, disassemble the blot sandwich, and carefully remove the membrane with blotting tweezers
- Let the membrane dry on a filter paper (store or proceed to immunodetection)