Western Blotting Protocol

References

1. Afreen, S., Riherd Methner, D. N., & Ferreira, A. (2017). Tau_{45-230} association with the cytoskeleton and membrane-bound organelles: Functional implications in neurodegeneration. *Neuroscience*, *362*, 104–117. https://doi.org/10.1016/j.neuroscience.2017.08.026
2. Lang, A. E., Riherd Methner, D. N., & Ferreira, A. (2014). Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. *Neuroscience*, *275*, 322–339. https://doi.org/10.1016/j.neuroscience.2014.06.017
3. Nicholson, A. M., Wold, L. A., Walsh, D. M., & Ferreira, A. (2012). β-Amyloid carrying the Dutch mutation has diverse effects on calpain-mediated toxicity in hippocampal neurons. *Molecular medicine (Cambridge, Mass.)*, *18*(1), 178–185. https://doi.org/10.2119/molmed.2011.00366

Solutions

1. Electrophoresis Buffer Stock: 125 mM Tris, 959 mM Glycine
2. Running Buffer: 16.6% (v/v) Electrophoresis Buffer Stock, 0.8% (v/v) 10% SDS
3. Transfer Buffer Stock: 10 mM Sodium Bicarbonate, 3 mM Sodium Carbonate
4. Transfer Buffer w/ Methanol: 10 mM Sodium Bicarbonate, 3 mM Sodium Carbonate, 20% Methanol
5. Transfer Buffer: 80% Transfer Buffer w/ Methanol, 16% Transfer Buffer Stock, 4% Methanol
6. Blocking Buffer: 50% PBS, 50% PBS-T, 5% (w/v) Carnation Nonfat Dry Milk

Protocol

SDS PAGE

1. Load previously prepared separating gel onto the running apparatus.
2. Cast and pour the stacking gel on top of the separating gel and load the comb.
3. Prepare samples by scraping in 2X Laemmli and boiling 10 minutes to homogenize.
4. When stacking gel has polymerized, pour in Running Buffer and remove the comb.
5. Load protein marker (20 µL) and samples into separate wells using a Hamilton syringe.
6. Run gel at 80 Volts for ~2 hours - watch for the dye to reach the bottom of the gel (don't run off!).

TRANSFER

1. Obtain a transfer box, sponge holder with sponges, 2 pieces of pre-cut absorbent paper, a pre-cut membrane, a stir bar, a tin pan, and a transfer top (match number with transfer box).
2. Rinse the membrane in 100% methanol for no more than 3 minutes.
3. Pour transfer buffer in the tin pan (until half full) and set the sponge holder in it- open, dark side down.

    a. Make sure one sponge is down and one is up.
4. Wet and lay down a piece of absorbent paper.
5. Remove gel from electrophoresis glass, cut off stacking gel, and rinse in transfer buffer before placing on top of absorbent paper.
6. Rinse the membrane briefly in transfer buffer and place on top of gel.
7. Put the second absorbent paper on top of membrane and close the sponge holder.
    a. Make sure there are no air bubbles between any two components of the "sandwich"!!
8. Put the stir bar in the transfer box and fill it to the minimum line with transfer buffer.
9. Place the sponge holder with your gel in it into the transfer box, dark side forward.
10. Put the transfer top on the transfer box, dark side forward.
11. Run the transfer at the appropriate amplitude for the time you wish the transfer to run.

BLOCKING and PRIMARY ANTIBODY INCUBATIONS

1. Prepare blocking buffer and stir 20 minutes with a stir bar or on rocker.
2. When transfer is finished, remove blot from transfer box to a pan with transfer buffer.
3. Cut the blot to the appropriate size and remove the marker using a razor blade.
4. If blocking buffer is ready, put directly into blocking buffer for 1 hour (use the amount you will need for the right primary antibody dilution).
    a. If blocking buffer is not ready, rotate blot in PBS until you can transfer into blocking buffer.
5. Add primary antibody directly to the blocking buffer containing the blot.
6. Incubate at room temperature for 30 minutes and put in the cold room overnight with rocking.

SECONDARY ANTIBODY INCUBATION and ECL DETECTION

1. Next morning, let blot incubate at room temperature with rocking for 30 minutes before rinsing.
2. Rinse the blot 3 x 15 minutes in PBST.
3. Block again for 1 hour.
4. Add secondary antibody to the blocking buffer containing the blot (1:1000).
5. Incubate at room temperature for 1 hour.
6. Rinse the blot 3 x 15 minutes in PBST.
7. ECL
    a. Pour off washing solution from blot and cover with ECL solution (1:1000 Hydrogen Peroxide in ECL solution), agitate 1 minute or less if bands begin to appear.
    b. Remove blot from ECL solution and cover with Saran Wrap, avoiding air bubbles.
    c. Expose blot using Quantity One Program and BioRad Imager Chemidoc XRS.

STRIPPING, DRYING, and STORAGE

1. After acquiring the image, place blot in stripping solution for 30 minutes at 58°C.
2. Wash the blot 3 x 5 min before either drying or re-blocking.
3. If re-blocking, proceed to BLOCKING and PRIMARY ANTIBODY INCUBATION, step #4.
4. If drying, remove the blot from the PBST and let dry on a piece of filter paper.

5. When dry, place another piece of filter paper on top, wait 5 minutes, and tape the filter paper pieces together and label.
6. Store at -80°C.
7. Then before blotting again, you must make the membrane hydrophilic by soaking 1 minute in methanol before blocking.