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Protocol status: Working
We use this protocol and it's working

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SOP for BCA protein assay and western immunoblotting

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ABSTRACT

SOP for BCA protein assay and western immunoblotting

MATERIALS

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SOLUTIONS:

- Tris buffered saline (TBS)
- TBS + 0.1% Tween-20 (TBST)
- Running Buffer

o 2.5mM Tris Base
o 19.2mM glycine
o 1% sodium dodecyl sulfate
o In MilliQ water
o pH should be around 8.4 but don't adjust

- Transfer Buffer (from 5X stock from Bio-Rad)
- Revert wash buffer

o 30% methanol
o 6.7% acetic acid
o In MilliQ water

- 5% nonfat dry milk in TBST
- MilliQ water
- Methanol

REAGENTS:

- Revert 700 Total Protein Stain (Li-Cor)
- Primary antibody(s)
- HRP-conjugated secondary antibody(s)
- Precision Plus Protein Dual Color ladder (fromBio-Rad)
- SuperSignal West PICO and FEMTO chemiluminescent substrates (ThermoFisher)

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BEFORE BEGINNING:

1 Ensure protein samples are prepared appropriately, using a BCA or other technique to measure the concentration and equalizing concentrations of samples. We use a BCA kit (ThermoFisher, #23225) according to the instructions and dilute the samples to around 2mg/mL with more lysis buffer (either RIPA or 1% SDS, whatever was used to extract the protein) and 1x Laemmli buffer.

EQUIPMENT:

2

2.1 4-20% polyacrylamide Criterion gels – 26 wells with 15uL/well (Bio-Rad)

2.2 Bio-Rad electrophoresis system

2.3 Midi size electrophoresis tank (Bio-Rad)

2.4 PVDF membrane (Bio-Rad)

2.5 Trans-Blot Turbo Transfer system (Bio-Rad)

2.6 Li-Cor Odyssey Fc imager

2.7 Appropriate containers for blot incubations, washes

2.8 Rocker that is safe in the refrigerator

2.9 One-sided razor blades

2.10 Transfer tools, including a fork to open the gel after electrophoresis and a roller to ensure contact between gel and membrane

2.11 Transfer stacks

2.12 Plastic sheet to aid in scrubbing bubbles out between Li-Cor plate and membrane

2.13 Flat forceps

2.14 Weigh boat

SDS-PAGE

3 Place gel cassette into tank and fill chamber to the fill line with running buffer. Fill cassette's reservoir too.

4 Dislodge any bubbles in wells by triturating in well with a micropipette.

5 Load appropriate volumes of samples and ladders. Load 4uL of ladder into the first well and 2uL into the last to maintain the orientation of the blot later.

6 Place lid on tank, ensuring black meets blank and red meets red!

7 Run electrophoresis at 60V for 1hr.

8 Run electrophoresis at 125V until finished, about 1hr 15min. Look for the dye front meeting the very bottom of the gel. TRANSFER:

TRANSFER:

9 Activate membrane with methanol for 60s.

10 Rinse membrane x2 in MilliQ water.

11 Equilibrate membrane and transfer stacks in cold transfer buffer.

12 Open gel cassette.

13 Trim wells and dye front off the top and bottom of the gel, respectively.

14 Float gel in a separate container of transfer buffer.

15 Put one transfer stack in the center of the transfer cell. Roll to ensure total contact with bottom of cell.

16 Lay membrane on top of the stack, rolling again to remove bubbles between layers.

17 CAREFULLY lay gel on top of membrane, ensuring the correct orientation of the gel and that all samples/ladders will be transferred to membrane. Roll gently to remove space between membrane and gel but not so aggressively that the gel is stretched.

18 Lay the second stack on top of the gel, rolling gently again.

19 Lock lid onto the cell. Pour out extra buffer and wipe any residual solution from the outside/bottom of the cell.

20 Run the Turbo Transfer system for 1 Midi gel using the preloaded Mixed Molecular Weight program. This runs for 7min.

21 Upon completion, open the cassette and remove the top stack and gel completely.

22 Add membrane to a container and rinse x2 with MilliQ water.

Total Protein Stain-For SIGNAL Normalization:

23 Add just enough Total Protein Stain to the membrane, about 10mL for a full Midi gel.

24 Cover with foil and rock for 5min at RT.

25 Rinse x2 with Revert Wash Buffer.

26 Place membrane on LiCor plate and remove air bubbles by placing the plastic sheet on top and scrubbing them out from under the membrane.

27 Remove plastic and image with the 700 channel for 2min. Save this image or re-image as needed to fix brightness/orientation issues.

IMMUNOBLOTTING:

28 Cut blot to strips of appropriate molecular weights using a razor blade, straight-edge guide of some kind, and clean, flat surface that can stand being cut into.

29 Place strips into 5% milk/TBST solution.

30 Block for 1hr at RT on a rocker.

31 During blocking, make up primary antibody solutions by adding primary antibodies at an appropriate dilution to 5% milk/TBST.

32 Pour off blocking solution and add primary antibody solution.

33 Incubate overnight at 4deg on a rocker.

34 Pour off antibody and wash 3x5min in TBST on a rocker.

35 Prepare secondary antibodies by diluting HRP-conjugated antibodies in 5% milk/TBST.

36 Incubate membranes in secondary antibody solutions for 1hr at RT on a rocker.

37 Pour off antibody and wash 3x5min in TBST on a rocker.

38 Wash at least once in TBS on a rocker to remove detergents ahead of developing. DEVELOPING & IMAGING:

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39 Add equal parts Pico Luminol/Enhancer and Pico Stable Peroxide to a weigh boat big enough for the membranes and mix.

40 Grab membranes with forceps and gently blot off excess TBS.

41 Place membrane into substrate solution. Ensure membrane is fully covered. Shake briefly to ensure the whole membrane gets treated.

42 Grab membrane and gently shake off excess substrate.

43 Place membrane onto Li-Cor plate and remove bubbles as before using the plastic sheet.

44 Remove plastic and image with the Chemi channel. Imaging times will be 30s for very highly abundant proteins or 2min for most proteins. Also enable the 700 channel for 30s to image the ladder.

45 If signal does not appear with the Pico substrate at a 2min imaging time, redo this section with the Femto substrate.

45.1 Try a diluted Femto solution by adding 1 part Femto Luminol/Enhancer, 1 part Femto Stable Peroxide, and 2 parts MilliQ water and repeat.

45.2 If a diluted Femto solution still provides dim signal, use equal parts Femto reagents without dilution.

45.3 For all images, make sure the 700 channel is imaged for 30s.