Western Blotting

Eric ECS Cordeiro-Spinetti

1University of Miami

ABSTRACT

It's a classic
1

Make RIPPA buffer (to be used in 3 months after adding Nuclease)

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mM Tris HCl, PH 7.4</td>
<td>5mL (1M stock)</td>
</tr>
<tr>
<td>150mM NaCl</td>
<td>5mL (3M stock)</td>
</tr>
<tr>
<td>1% Triton X-100 or NP-40</td>
<td>1 mL</td>
</tr>
<tr>
<td>0.1% SDS</td>
<td>1mL (10% supply center solution)</td>
</tr>
<tr>
<td>1mM EDTA</td>
<td>0.2 mL (0.5M stock)</td>
</tr>
<tr>
<td>10mM Naf</td>
<td>0.042g (@L5P1 chemical room)</td>
</tr>
<tr>
<td>0.5% Sodium deoxycholate</td>
<td>0.5g (@L5P1 chemical room)</td>
</tr>
</tbody>
</table>

Add ddH2O to 100 mL
Add PMSF [final] = 1mM and any other protease inhibitors immediately before use
Add 1μL Pierce™ Universal Nuclease per 1mL

2

SDS Running Buffer

Cell lysis
3 Trypsinize cells

4 Wash pellet with PBS

5 Remove all supernatant with micropipette

6 Freeze pellet in liquid N2

7 Thaw quickly at \(37 \, ^\circ\text{C}\)

8 Resuspend cell pellet on buffer 100 µL

9 Vortex 00:00:15

10 Incubate on Ice 00:20:00

11 Vortex 00:00:15
12 Collect supernatant

13 Centrifuge 10000 RPM for 00:05:00

14 Collect supernatant without disturbing the pellet

15 Quantify protein

15.1 799 uL water + 1 uL lysate 
      + 200 uL 5x Bradford reagent

15.2 Read at 595 nm

16 Load 50 µg of protein/well

17 Loading buffer 50 µL β-mercaptoethanol + 950 µL 2x Laemli Buffer
18. Water up to 15 µL

19. Boil at 95 °C before loading 00:00:15

20. Add 10 µL of ladder (Precision Plus Protein - Dual Color Standards)

21. Run at 120-150 volts 01:00:00

22. Activate PVDF membrane in methanol

23. Soak filter paper in Towbin Buffer

24. Equilibrate gel in Towbin buffer 00:10:00

25. Semi-dry transfer
25.1  90’ (<50KDa) or 120’ (>50KDa)

25.2  Mount sandwich (paper-membrane-gel-paper)

25.3  45 mA/gel (constant current)

26  Cut out LEFT UPPER corner of the membrane

27  Block membrane with 5% BSA in PBS 0.1% Tween (PBST) for 00:30:00

28  Add primary antibody in BSA-PBST and incubate overnight 18:00:00 at 4 °C

29  Wash 3x with PBST for 00:05:00

30  Add HRP secondary antibody in BSA-PBST and incubate for 01:00:00 at room temperature on a shaker
31 Wash 3x with PBST for 00:05:00

32 Add 500 µL ECL (enhanced chemiluminescent) per membrane strip