مؤسسة مستشفى سرطان الأطفال-مصر
57357
Children's Cancer Hospital Foundation - Egypt

Proteomics & Metabolomics Unit
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https://www.57357.org/proteomics-unit/

A. Reagents Preparation

Transfer Buffer A
[0.3M Tris, 5% Methanol]
30 ml 1M Tris
5 ml Methanol
Complete to 100ml MilliQ

Transfer Buffer B
[25mM Tris, 5% Methanol]
2.5 ml 1M Tris
5 ml Methanol
Complete to 100ml MilliQ

Transfer Buffer C
[25mM Tris, 5% Methanol]
2.5 ml 1M Tris
5 ml Methanol
0.52g 6-aminocapronic acid
Complete to 100ml MilliQ

10x TBS:
24 g Tris base
88 g NaCl
700 ml MilliQ
Adjust **pH 7.6**
Complete to 1L MilliQ

1x TBST: (0.1% Tween)
100 ml 10x TBS
900 ml MilliQ
1 ml Tween 20

Blocking Solution (Milk):
5% non-fat milk in TBST

Blocking Solution (BSA):
5% BSA in TBST

Medium Stripping Solution:
15 g Glycine
1 g SDS
10 ml Tween 20

High Stripping solution:
20 ml 10% SDS
12.5 ml Tris HCl pH 6.8
67.5 ml MilliQ
0.8 ml BME

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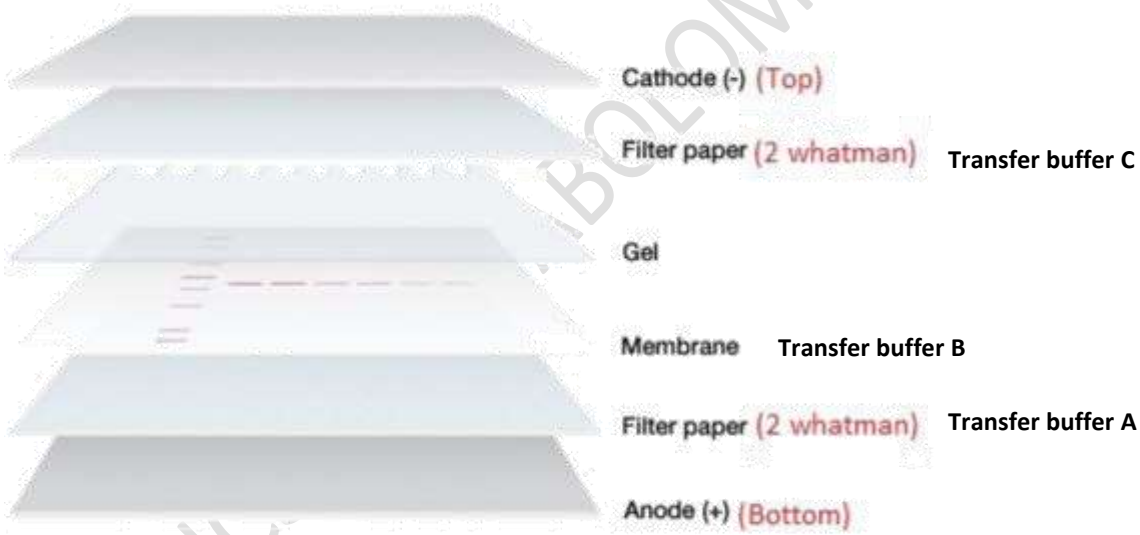
B. **Transfer:**

1. Soak 2 whatman filter papers in **transfer buffer A** (Bottom) for 20 min.
2. Soak 2 whatman filter papers in **transfer buffer C** (Top) for 20 min.
3. Soak membrane in methanol in 30 sec. Discard. Then soak in **transfer buffer B** for 30 min while shaking.
   (You could change the transfer buffer one time to make sure methanol was washed)
4. Wash the gel (10%) with **transfer buffer B** after removing it from the cassette using pipette.
5. Assemble the transfer sandwich in the semi-dry trans-blotter as follows:



6. Close the trans-blotter and run at 100mA constant, 25V limit, 40 minutes, using BioRad Semi-Dry Blotter*.
   *Run conditions may be adjusted depending on the target protein.

C. **Immunoblotting part I:**

7. Stain the gel in coomassie stain for ~ 20 min. Then remove stain and add destain solution
8. Wash the membrane for 2 min in TBST while shaking.
9. Prepare either 5% non-fat dry milk in TBST solution or 5% BSA in TBST solution (depending on the used antibody).

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10. Remove the TBST solution from the membrane.
11. For **Blocking**: Incubate membrane in 5% non-fat dry milk or 5% BSA for 1 hour at room temperature with shaking.
12. Prepare 1-3 ml of primary antibody dilution in either 3% non-fat dry milk or 3% BSA.
    (Volume depends on the length of the membrane, make sure to use enough amounts to cover the membrane).
13. Remove the blocking solution from the membrane and incubate the membrane in primary antibody solution overnight at 4°C with shaking.

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D. **Immunoblotting part II:**

1. Remove primary antibody solution.
2. Wash membrane with 1X TBST 3 times, 15 minutes each.
14. Prepare 1-3 ml secondary antibody dilution in either 3% non-fat dry milk or 3% BSA.
    (Volume depends on the length of the membrane, make sure to use enough amounts to cover the membrane).
3. Remove TBST from the membrane and incubate the membrane in secondary antibody solution at room temperature for 1 hour with shaking.

E. **Detection:**

1. Remove secondary antibody solution.
2. Wash membrane with 1X TBST 3-4 times, 5 minutes each.
3. Prepare ECL solution by mixing both components at a ratio of 1:1 prior to detection (~2 ml per membrane).
4. Drain TBS from the membrane and incubate the membrane in ECL solution for 5 minutes at room temperature.
5. Drain excess ECL and visualize bands using chemidoc detection system.