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

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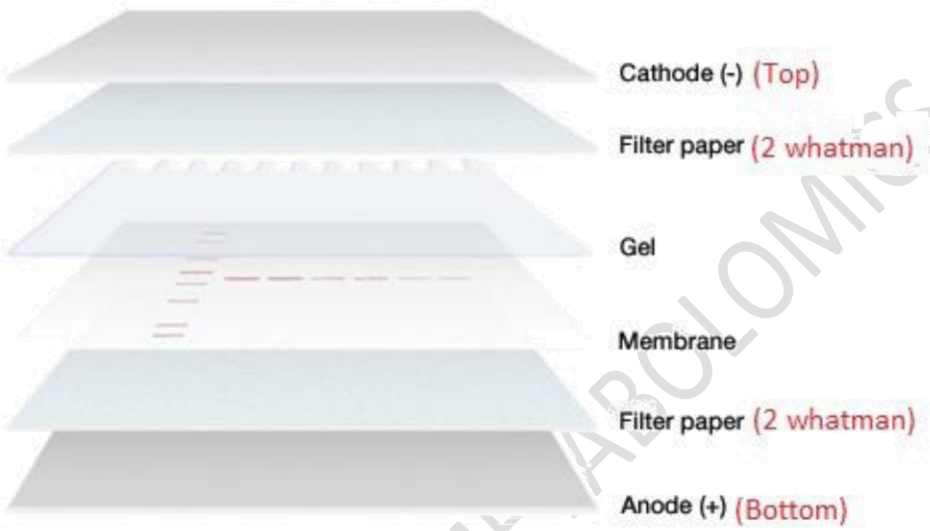
Western Blotting part I

a. Transfer:

1. Soak 4 whatman filter papers in transfer buffer.
2. Soak membrane in methanol
3. Assemble the transfer sandwich in the semi-dry trans-blotter as follows:



4. Close the trans-blotter and run at 400mA for 40 minutes.

b. Immunoblotting part I:

5. Prepare 5% non-fat dry milk in TBST solution.
6. Incubate membrane in 5% non-fat dry milk for 1 hour at room temperature with shaking.
7. Prepare 4 ml of primary antibody dilution in either 5% non-fat dry milk of 5% BSA as indicated in the antibody's data sheet.
8. Drain the milk from the membrane and incubate the membrane in primary antibody solution overnight at 4°C with shaking.

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Western Blotting part II

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a. Immunoblotting part II:

1. Drain primary antibody solution.
2. Wash membrane with 1X TBS 3 times, 5 minutes each.
3. Prepare secondary antibody dilution as indicated in its data sheet in 5% non-fat dry milk solution
4. Drain TBS from the membrane and incubate the membrane in secondary antibody solution at room temperature for 1 hour with shaking.

b. Detection:

1. Drain secondary antibody solution.
2. Wash membrane with 1X TBS 3 times, 5 minutes each.
3. Prepare ECL solution by mixing both components at a ratio of 1:1 prior to detection.
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.