SEVEN HILLS BIOREAGENTS

Western Blotting: Non-Reduced Protocol

Buffers:

10X Tris-Tricine Buffer : 1.0 M Tris-Base, 1.0 M Tricine, 1% SDS
Anode Buffer: 300 mM Tris-Base, 35% Methanol
Cathode Buffer: 26 mM Tris-Base, 40 mM Aminocaproic Acid, 20% Methanol, 0.1% SDS, pH 9.4
TBS-T: TBS with 0.05% Tween-20

Protocol:

Note that this protocol provides methods for semi-dry transfer of proteins

1. Dilute protein in Laemmli sample buffer **without** β-mercaptoethanol.
2. Heat protein samples at 100°C for 4 minutes.
3. Run protein samples for 1.5 hours at 125V, 55mA on 10-20% Tricine Gel with 1X Tris-Tricine Buffer
4. Transfer onto **0.1 μm** nitrocellulose membrane: 1 hour at 180 mA, 36V with anode & cathode buffers.
5. Rinse membrane with TBS-T 3 times, 10 minutes each at RT (room temperature), with rocking.
6. Block membrane in 5% Non-Fat Dry Milk in TBS-T, 1hour at RT, with rocking.
7. Rinse membrane with TBS-T 3 times, 10 minutes each at RT, with rocking.
8. Dilute the primary antibody in TBS-T and incubate membrane with primary antibody overnight at 4°C or RT, with rocking.
9. Rinse membrane with TBS-T 3 times, 10 minutes each with rocking at RT.
10. Dilute secondary antibody in TBS-T and incubate membrane for 1 hour at RT, with rocking.
11. Rinse membrane with TBS-T 3 times, 10 minutes each at RT, with rocking.
12. Develop blot with Pierce SuperSignal WestPico Chemiluminescent Substrate.