Western Blot

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This protocol is used to western blot proteins-of-interest.

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1 Add 4X Laemmli buffer to protein samples and incubate at 95^{o}C for 7 minutes. 7m

2 Load samples into 12% polyacrylamide gel and run in running buffer at 80-100V for 60-120 minutes. 2h

3 Transfer gel to 0.2µm nitrocellulose membrane at 350mA for 60 minutes at 4^{o}C. 1h

4 Wash membrane with 1X TBS-T then block in 10% milk for 30 minutes at room temperature. 35m

1X TBS-T: 10mM Tris-HCl + 150mM NaCl + 0.5% Tween-20 in H_2O, pH 8.0

5 Wash membrane with 1X TBS-T for 4x 5 minutes. 20m

6 Incubate membrane in primary antibody solution overnight at 4^{o}C. 1d
Antibodies diluted to the appropriate concentration in 2% bovine serum albumin in 1X TBS-T with 0.02% sodium azide

7 Wash membrane with 1X TBS-T for 4x 5 minutes. 20m

8 Incubate membrane in secondary antibody solution for 1 hour at room temperature. 1h
Secondary antibody (horseradish peroxidase conjugated) is diluted in 10% milk.

9 Wash membrane with 1X TBS-T for 4x 5 minutes. 20m

10 Rinse ECL Clarity Solution over membrane 20x then image. 1h

11 Once imaging is complete, wash membrane with 1X TBS-T x2 then H_2O x2 then let dry at room temperature overnight before storing. 10m

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