proteintech®
Antibodies | ELISA kits | Proteins

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# PRODUCT-SPECIFIC PROTOCOLS

## WESTERN BLOT (14482-1-AP)

| Sample type | Amount of protein loaded | Membrane | Transfer type | Blocking buffer | Primary antibody dilution | Incubation time | Secondary antibody | Incubation time | Detectionmethod |
|---|---|---|---|---|---|---|---|---|---|
| THP-1 cells | 30ug | PVDF | Wet | 5% normal non-fat milk+TBST | 1:5000 | 37°C 1 hour | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |
| PC-12 cells | 40ug | PVDF | Wet | 5% milk in TBST | 1:600 | Room temperature 1.5 hours | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |
| HL-60 cells | 50ug | PVDF | Wet | 5% milk in TBST | 1:500 | Room temperature 1.5 hours | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |
| mouse brain tissue | 30ug | PVDF | Wet | 5% normal non-fat milk+TBST | 1:5000 | 37°C 1 hour | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |
| U-937 cells | 30ug | PVDF | Wet | 5% normal non-fat milk+TBST | 1:3000 | 37°C 1 hour | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |

# PROTOCOL

1. Prepare sample lysate, heat lysate in sample buffer at 100°C for 5min, and resolve proteins via SDS-PAGE. (Note: Prepare sample lysate without heating or heating at 37°C accroding to picture recommended for some membrane proteins.)
2. Transfer proteins from the gel onto the membrane.
3. Incubate membrane with Blocking buffer on a rocking platform.
4. Prepare the primary antibody in Blocking buffer.
5. Incubate membrane with primary antibody on a rocking platform.
6. Wash the membrane 3 times for 10 minutes each in 1X TBST
7. Prepare the secondary antibody in blocking buffer.
8. Incubate the membrane with secondary antibody on a rocking platform.
9. Wash the membrane 3 times for 10 minutes each in 1X TBST.
10. Incubate the membrane with Chemiluminescent-HRP substrate according to the manufacturer's instructions.
11. Expose the membrane to autoradiography film or another detection system for the appropriate time period that yields best results. For best results, expose for 30-300 sec.

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