proteintech®
Antibodies | ELISA kits | Proteins

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# PRODUCT-SPECIFIC PROTOCOLS

## WESTERN BLOT (11147-2-AP)

| Sample type | Amount of protein loaded | Membrane | Transfer type | Blocking buffer | Primary antibody dilution | Incubation time | Secondary antibody | Incubation time | Detectionmethod |
|---|---|---|---|---|---|---|---|---|---|
| EC109 cells | 30ug | PSQ | Semi-dry | 5% milk in TBST | 1:500 | 4°C over night | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |
| DC2.4 cells | 40ug | PSQ | Semi-dry | 5% milk in TBST | 1:200 | Room temperature 1.5 hours | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |
| HEK-293 cells | 30ug | PSQ | Semi-dry | 5% milk in TBST | 1:400 | 1.5 h at room temp | HRP conjugated anti-Rabbit IgG (H+L) | 37°C 1 hour | ECL |

# PROTOCOL

1. Prepare sample lysate for protein with higher MW(>150 kDa). Add the fresh DTT into the sample lysate to a final concentration of 100 mM just before heating. Heat sample lysate at 70°C for 10 min. Load the sample lysate into the Tris-acetate gradient gel.
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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