proteintech®
Antibodies | ELISA kits | Proteins

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PRODUCT-SPECIFIC PROTOCOLS

WESTERN BLOT (22641-1-AP)

Sample type	Amount of protein loaded	Membrane	Transfer type	Blocking buffer	Primary antibody dilution	Incubation time	Secondary antibody	Incubation time	Detectionmethod
A375 cells	40ug	PSQ	Semi-dry	5% milk in TBST	1:1000	Room temperature 1.5 hours	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
A549 cells	30ug	PSQ	Semi-dry	5% milk in TBST	1:300	Room temperature 1.5 hours	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
HEK-293T cells	30ug	PSQ	Semi-dry	5% milk in TBST	1:300	Room temperature 1.5 hours	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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