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Antibodies | ELISA kits | Proteins

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PRODUCT-SPECIFIC PROTOCOLS

WESTERN BLOT (19218-1-AP)

Sample type	Amount of protein loaded	Membrane	Transfer type	Blocking buffer	Primary antibody dilution	Incubation time	Secondary antibody	Incubation time	Detectionmethod
A431 cells	20ug	PVDF	Wet	5% milk in TBST	1:4000	37°C 1 hour	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
human skeletal muscle tissue	30ug	PVDF	Wet	5% milk in TBST	1:300	1.5 h at room temp	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
HepG2 cells	30ug	PVDF	Wet	5% milk in TBST	1:500	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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