proteintech®
Antibodies | ELISA kits | Proteins

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PRODUCT-SPECIFIC PROTOCOLS

WESTERN BLOT (25124-1-AP)

Sample type	Amount of protein loaded	Membrane	Transfer type	Blocking buffer	Primary antibody dilution	Incubation time	Secondary antibody	Incubation time	Detectionmethod
MCF-7 cells	30ug	PVDF	Wet	5% milk in TBST	1:600	1.5 h at room temp	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
T-47D cells	40ug	PVDF	Wet	5% milk in TBST	1:2000	Room temperature 1.5 hours	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
A431 cells	30ug	PVDF	Wet	5% milk in TBST	1:600	1.5 h at room temp	HRP conjugated anti-Rabbit IgG (H+L)	37°C 1 hour	ECL
HEK-293 cells	30ug	PVDF	Wet	5% milk in TBST	1:600	1.5 h at room temp	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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