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Antibodies | ELISA kits | Proteins

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PRODUCT-SPECIFIC PROTOCOLS
WESTERN BLOT (pabr1)

Sample type	Amount of protein loaded	Membrane	Transfer type	Blocking buffer	Primary antibody dilution	Incubation time	Secondary antibody	Incubation time	Detection method
Transfected HEK-293 cells	30ug	nitrocellulose	Semi -dry	5% milk in PBST	1:1000	4°C overnight	HRP conjugated anti-Rabbit IgG (H+L)	1 hour at RT	ECL

PROTOCOL

1. Prepare sample lysate, heat lysate in sample buffer at 95°C for 5 min and resolve proteins via SDS-PAGE. (Note: Try preparing sample lysate without heating or heating at 37°C 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 1XTBST.
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 1XPBST.
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 1-10 sec (this will depend on your secondaries and ECL).