FOR LAB RESEARCH USE ONLY NOT FOR USE IN HUMANS OR ANIMALS

MATERIAL SAFETY DATA SHEET (MSDS)

Protocol for Immunoblotting and Immunoprecipitation:

Preparation of Cell Lysate:

Add RIPA buffer to cells (100µL to a 35 mm dish, 200µL to a 60 mm dish, 500µL to a 100 mm dish) while the culture dish is placed on ice. Scrape the cells and gently rock the suspension on either a rocker or an orbital shaker in the cold room for 15 minutes to lyse cells. **Sonicate** in ice water with bath sonicator until the sample is no longer viscous. Centrifuge the cells at 12000g at 4℃ for 5 minutes to remove pellet. Move the supernatant to a fresh tube. Final concentration of cell lysate will be 2-3 µg/µL. For WB, add 4×SDS stop buffer to the lysate to a 1xSDS final concentration.

Immunoblotting:

After boiling samples for 10 minutes, load the 20-50 µ L samples onto SDS-PAGE gel. For most proteins, **30-80 µ g total lysate proteins per lane** loading amount is suggested, because 80% of protein species in cells are at very low concentrations. Soak the gel in western blot transfer buffer. **PVDF membrane is recommended for most proteins.** Dip the membrane into methanol for 1-2 minutes, soak the membrane in transfer buffer for 10 minutes and place on a thick stack of buffer-soaked filter paper. Cover with another stack of buffer-soaked filter papers. Cover up the transfer apparatus. Gel should be on the negative side of the membrane. Run for 90 minutes at current of 1 mA per cm². Incubate the membrane in blocking buffer for 1 hour at room temperature or overnight at 4℃. Wash membrane 3×5 minutes with washing buffer. Dilute primary antibody with blocking buffer. Incubate the membrane for **1.5 hours at room temperature.** Wash membrane 3×5 minutes with washing buffer. Dilute secondary antibody with blocking buffer. Incubate the membrane for 1 hour at room temperature. Wash membrane 3×5 minutes with washing buffer. Wash membrane 3×10 minutes with washing buffer. Develop color with ECL.

Immunoprecipitation:

Transfer 200-350µL cell lysate (containing 1-3 mg total protein) to the microfuge tube. Add 150-300µL incubation buffer and 1-4µg primary antibody to the cell (or pre-cleared) lysate. Optimal antibody concentration should be determined by titration. Set up a negative control experiment with control IgG (corresponding to the primary antibody source). Gently rock the incubations at 4℃ for 2-4 hours or overnight. Add 50µL Protein A or G sepharose beads slurry to capture the immunocomplex. Gently rock the mixture at 4℃ or 1-4 hours. Take off the end caps, the supernatant is released from the bottom of the spin columns. If necessary, resuspend the beads mixture to enhance the flow velocity. Wash the beads 5 times with 1×TBST containing 1×Protease inhibitor. After the final washing step centrifuge the spin columns at 500 g for 30 seconds at 4℃ and collect the supernatant with collection tubes and discard.
Place Spin Columns in a fresh microfuge tube and pool the elutions. Elute the pellet with 40µl Elution buffer and centrifuge at 8000-10000g for 1 min at 4℃. Repeat once for the elution with new 40µl elution buffer. Add 10µl Alkali neutralization buffer and 30µl 4×Sample buffer to the elutions, heat at 95-100℃ for 5 minutes. Store at -20℃ or load 20-40µl on SDS-PAGE and detect by WB.

Protocol for Immunofluorescence with Tissue Culture Cells:

Culture the cells on small round cover glass. Fix cells with 4% PFA or organic solvents for 20 minutes at 4℃ (**If you choose to fix your samples with organic solvents, skip the next step and proceed directly to blocking step**). Transfer the glass into 0.2% Triton X-100-PBS solution for exactly 5 minutes. Block the cells with BSA-PBS solution for 1h at room temperature or overnight at 4℃. Add 50µL of specific primary antibodies on cover glass and incubate for 1 hour at room temperature in a moist container. Wash the cover glass 3 times with PBS. Add 50µL of fluorescent-labeled secondary antibody on each cover glass and incubate for 1 hour at room temperature in a moist container in the dark. Mount the coverslip in 15% glycerol or a small volume of hydromount on a slide. Seal the edge of the coverslip.

Protocol for Immunohistochemistry on Paraffin-Embedded Tissue Sections:

Deparaffinize slides in 2 changes of xylene, one for 40 minutes, the other for 20 minutes.
Transfer slides to 100% alcohol, 95% alcohol, 80% alcohol, 60% alcohol, and ddH_2O for 5 minutes each. Place slides in a vessel filled with antigen retrieval buffer and microwave on medium power for several minutes (700 W oven), allow retrieval solution to cool at room temperature. Rinse 2×5 minutes with TBS. Block endogenous peroxidase activity by incubating sections in 3% H_2O_2 solution in methanol for 30 minutes. Rinse 2×5 minutes with TBS. Block in 5-10% goat serum in TBS for 30 minutes at room temperature. Drain slides for a few seconds (do NOT rinse) and wipe around the sections. Apply primary antibody made up in TBS. Incubate for 1 hour at room temperature or 4℃ overnight. Rinse 2×5 minutes with TBST. Apply secondary biotinylated antibody made up in TBS for 1 hour at room temperature. Rinse 2×5 minutes with TBS. Apply streptavidin peroxidase for 15 minutes or 30 minutes at room temperature. Rinse 2×5 minutes with TBS. Develop with chromogen (DAB) at room temperature, watching under microscope. Rinse in running tap water for 5 minutes. Counterstain in Mayer's hematoxylin bath for 30-60 seconds. Wash in water bath 7-8 times, then with tap water for 3 minutes. Dehydrate through 60%, 80%, 95%, and 100% alcohol for 5 minutes each. Transfer to xylene for 5 minutes. Air-dry for 30 minutes. Mount slides.

Buffers

10 x PBS	**2000 mL**
H_2O	1000 mL
27 mM KCl	4 g
14 mM KH_2PO_4	4 g
43 mM $Na_2HPO_4 \cdot H_2O$	22.9 g
1.37 M NaCl	160.0 g
ddH_2O up to 2 liters	Filter and Autoclave

RIPA buffer	**1000 mL**
50mM Tris HCL, pH 7.4	50ml
150mM NaCl	8.76 g
1% Triton X-100	10ml
0.5%Sodium deoxylcholate	5 g
0.1%SDS	1 g
1mM EDTA (0.5M stock)	2ml
10mM NaF	0.42 g
ddH20 to 1000ml	
1mM PMSF (add before use)	0.17 g

Transfer buffer	**1000 mL**
48mM Tris-base	5.81 g
39 mM Glycine	2.93 g
0.0375% SDS	0.375 g
20% Methanol	200ml
ddH20 to 1000ml	

Washing buffer TBST	1000 mL
20mM Tris-base	2.4 g
150mM NaCl	8.7 g
0.2% Tween-20	2mL
Adjust pH to 7.6	
ddH2O to 1000 mL	

Blocking buffer	**100 mL**
5% Non-fat Milk	5 g
Washing buffer TBST pH 7.6	100 mL

TBS	**1000 mL**
20 mM Tris base	2.4g
150 mM NaCl	8.7 g
ddH_2O	1000 mL
Adjust pH to 7.6 with HCl	

TRIS-EDTA	**1000 mL**
10mM Tris base	1.21 g
1 mM EDTA	0.37 g
Adjust pH to 9.0	
ddH2O to 1000mL	

Antigen retrieval buffer	**1000mL**
10mM Tri-sodium citrate 2H20	2.9 g
1.9mM Citric acid H20	0.4 g
Adjust pH to 6.0	
DdH20 to 1000ml	

IP Elution buffer	**1000 mL**
150mM Glycine	11.26 g
500 mM NaCl	29.2 g
Adjust pH to 1.5-2.0 with HCL	
ddH2O to 1000 mL	

Alkali Neutralization buffer	**1000 ml**
20mM NaH2PO4	2.8g
1.4M NaCl	80g
ddH20 to 1000ml	
Add NaH2PO4 to saturation	Approx. 200g

IP incubation buffer	**1000 mL**
1 x PBS	1000 mL
5mM EDTA	1.46 g
10mM NaF	0.42 g
Adjust pH to 7.4	

BSA-PBS solution	**100 mL**
1 x PBS	100mL
5% BSA	5g

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PRODUCT IDENTITY:

Purified polyclonal and monoclonal antibodies.

INGREDIENT COMPOSITION/INFORMATION:

Glycerol	50%	Cas#56-81-5
Water	40-50%	Cas#7732-18-5
Animal proteins	10-50%	
Sodium Chloride	<1%	Cas#7647-14-5
Sodium Phosphate, Dibasic	<1%	Cas#7558-79-4
Sodium Azide	<0.1%	Cas#26628-22-8

The only hazardous substance present in these products is sodium azide, in very small amounts as an antimicrobial preservative.

HAZARDOUS IDENTIFICATION: Overview of hazardous ingredients:

Materials: Sodium azide - less than or equal to 0.1% (w/v). Toxicity Data LD50=27 mg/kg (oral-rat).
This product is considered non-hazardous under 29 CFR 1910.1200 (Hazard Communication).
Physical state of sodium azide-containing antibody: Odorless, light gold color, clear liquid.

FIRST AID MEASURES:

Skin Contact	Wash off skin thoroughly with soap and water. Remove contaminated clothing and wash before reuse.
Eye Contact	Ensure adequate flushing of eye contamination for at least 15 minutes.
Inhalation	Remove from exposure, rest and keep warm.
Ingestion	Wash mouth out thoroughly with water and drink plenty of water.

FIRE FIGHTING MEASURES:

Suitable extinguishing media: Water Spray, Carbon Dioxide, Dry Chemical Powder, or appropriate foam.

ACCIDENTAL RELEASE MEASURES:

Wear appropriate protective clothing. Mop up with an absorbent cloth and arrange removal by a disposal company. Wash site of spillage thoroughly with water and detergent.

HANDLING AND STORAGE:

Use only in area provided with exhaust ventilation. Avoid contact with eyes. Wear a lab coat.
Keep container tightly closed, and in a cool and well-ventilated area.
Store at -20℃.

EXPOSURE CONTROLS/PPE:

Follow good laboratory practice when handling this product. The following precautionary measures are recommended:
- Eye protection: Laboratory safety goggles.
- Hands: Chemical resistant gloves.
- Skin: Laboratory protective clothing.
- Respiratory: Fume hood or in areas with adequate ventilation.

PHYSICAL AND CHEMICAL PROPERTIES:

General Information: Form – Liquid.

CHEMICAL STABILITY AND REACTIVITY:

Stability: Stable.
Conditions to avoid: None known.
Incompatibility: None known.
Hazardous Polymerization: Will not occur.

TOXICOLOGICAL INFORMATION:

May be harmful if inhaled and absorbed through skin.
May be harmful if swallowed.
May be irritating to mucous membranes and upper respiratory tract.
May cause eye/skin irritation.
Carcinogenic effects: Not available.
Mutagenic effects: Not available.
Reproduction toxicity: Not available.
Teratogenic effects: Not available.

ECOLOGICAL INFORMATION:

Data not yet available.

DISPOSAL CONSIDERATIONS:

In accordance with local and national regulations.

TRANSPORT INFORMATION:

Not classified as dangerous in the meaning of transport regulations.

PRECAUTIONS:

Store the antibodies at -20℃.
The above information is believed to be correct but should only be used as a guide for experienced personnel. Proteintech Group Inc. will not be liable for any damage resulting from the handling of or from contact with the above product.

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