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Antibodies | ELISA kits | Proteins

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General Protocols

Protocol for Immunoblotting and Immunoprecipitation:

Preparation of Cell Lysate:

Add RIPA buffer to cells (100 µL to a 35 mm dish, 200 µL to a 60 mm dish, 500 µL 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 12000 g 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 1×SDS final concentration.

Immunoblotting:

1. After boiling for 10 minutes, 20-50 µL sample will be loaded to SDS PAGE. For most proteins, **30-80 µg total lysate proteins per lane** loading amount is suggested, because 80% of protein species in cells are at very low concentration.
2. 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 it on a thick stack of buffer-soaked filter paper. Then cover it with another stack of buffer soaked filter papers. Cover up the transfer apparatus. Gel should be on the negative side of the membrane.
3. Run for 90 minutes at current of 1 mA per cm^2.
4. Incubate the membrane in blocking buffer for 1 hour at room temperature or overnight at 4℃ . Wash membrane 3×5 minutes with washing buffer.
5. Dilute primary antibody with blocking buffer. Incubate the membrane **for 1.5 hours at room temperature.** Wash membrane 3×5 minutes with washing buffer.
6. Dilute secondary antibody with blocking buffer. Incubate the membrane for 1 hour at room temperature. Wash membrane 3×5 minutes with washing buffer.
7. Wash membrane 3×10 minutes with washing buffer.
8. Develop color with ECL.

Immunoprecipitation:

1. Transfer 200-350 µL cell lysate (contain 1-3 mg total protein) to the microfuge tube.
2. Add 150-300 µL incubation buffer and 1-4 µg 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.
3. Add 50 µL Protein A or G sepharose beads slurry to capture the immunocomplex. Gently rock the mixture at 4℃ for 1-4 hours.
4. Take off the End caps, the supernatant is released from the Spin Columns bottom If necessary, resuspend the beads mixture to enhance the flow velocity.
5. Wash the beads 5 times with 1×TBST containing 1×Protease inhibitor. After last washing, centrifuge the Spin Columns at 500 g for 30 seconds at 4℃ and collect the supernatant with Collection Tubes and discard it.
6. Place Spin Columns in a fresh microfuge tube and pool the elutions. Elute the pellet with 40 µl Elution buffer and centrifuge at 8000-10000g for 1 min at 4℃ , Repeat once for the elution with new 40 µl Elution buffer .
7. Add 10µl Alkali neutralization buffer and 30µl 4×Sample buffer to the elutions, heat at 95-100℃ for 5 minutes. Store it at -20℃ or load 20-40 µl on SDS-PAGE and detect it by WB.

Protocol for Immunofluorescence with Tissue Culture Cells:

1. Culture the cells on small round cover glass.
2. Fix cells with 4% PFA or organic solvents for 20 minutes at 4℃
   (If you use organic solvents fixed, just skip step 3 and proceed directly to step 4).
3. Transfer the glass into 0.2% Ttiton X-100-PBS for exact 5 minutes.
4. Block the cells with BSA-PBS for 1h at room temperature or overnight at 4℃ .
5. Add 50 µL of specific primary antibodies on cover glass and incubate for 1 hour at room temperature in a moist container.Wash the cover glass for three times with PBS.
6. Add 50 µL of fluorescence-labeled secondary antibody on each cover glass and incubate for 1 hour at room temperature in a moist container in dark.
7. Mount the coverslip in 15% glycerol or a small volume of hydromount on a slide.
8. Seal the coverslip with nail polish on the edge.

Protocol for Immunohistochemistry on Paraffin-Embedded Tissue Sections:

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

Buffers

10 x PBS	2000 mL
H_2O	1000 mL
KCl	27 mM
KH_2PO_4	14 mM
$Na_2HPO_4 \cdot H_2O$	43 mM
NaCl	1.37 M
ddH_2O up to 2 liter	Filter and Autoclave

RIPA buffer	1000 mL
Sodium Chloride	150 mM
NP-40 or Triton X-100	1.0%
Sodium Deoxycholate	0.5%
SDS	0.1%
Tris base	50 mM
EDTA	5 mM
EGTA	1 mM
Sodium Fluoride	5 -10 mM
Sodium Orthovanadate	1 mM
PMSF (add before use)	1 mM

Transfer buffer	1000 mL
Tris base	48 mM
Glycine	39 mM
SDS	0.037%
Methanol	20%

Washing buffer TBST	
Tris-HCl	20 mM
NaCl	150 mM
KCl	5 mM
Tween-20	0.2%
pH	7.4

Blocking buffer	
Non-fat Milk	5 g
Washing buffer TBST pH 7.4	100 mL

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

Tris-EDTA	1000 mL
Tris base	10 mM
EDTA	1 mM
pH	9.0

10×Citrate Buffer	500 mL
Tri-sodium citrate (dihydrate)	15 g
Citric acid (anhydrous)	2 g
ddH_2O	500ml
pH	6.0

Proteintech Group, USA,
5400 Pearl Street, Suite 300,
Rosemont, IL 60018, USA
t 1-888-478-4522
f 1-312-4558408

Proteintech Europe,
Manchester Science Park, Kilburn House,
Lloyd Street North, Manchester, M15 6SE
t (+44)-161-22-66-144
f (+44)-161-23-21-272

San Ying Biotechnology, China,
D3-3, No.666 Gaoxin Avenue, Wuhan East Lake
Hi-tech Development Zone, Wuhan, P.R.C.
t 86-27-87531629
f 86-27-87531627

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MATERIAL SAFETY DATA SHEET (MSDS)

PRODUCT IDENTITY:

Purified polycolonal and monoclonal antibodies.

COMPOSITION/INFORMATION ON INGREDIENTS:

Glycerol	50%	Cas#56-81-5
Water	40-50%	Cas#7732-12-5
Animal proteins	10-50%	
Sodium Chloride	<1%	Cas#7647-14-5
Sodium Phosphate, Dibasic	<1%	Cas#7558-79-4
Sodium Azide	<0.1%	Cas#26628-2-8

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

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 none 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 ventilations.

PHYSICAL AND CHEMICAL PROPERTIES:

General Information: Form – Liquid.

CHEMICAL STABILITY AND REACTIVITY:

Stability: Stable under recommended storage conditions.

Conditions to avoid: Heat and moisture.

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 only used as a guide for experienced personnel. Proteintech Group Inc. shall not be liable for any damage resulting from the handling or from contact with the above product.

For lab use only, not for diagnostic or therapeutic work.

Proteintech Group, USA,
5400 Pearl Street, Suite 300,
Rosemont, IL 60018, USA
t 1-888-478-4522
f 1-312-4558408

Proteintech Europe,
Manchester Science Park, Kilburn House,
Lloyd Street North, Manchester, M15 6SE
t (+44)-161-22-66-144
f (+44)-161-23-21-272

San Ying Biotechnology, China,
D3-3, No.666 Gaoxin Avenue, Wuhan East Lake
Hi-tech Development Zone, Wuhan, P.R.C.
t 86-27-87531629
f 86-27-87531627