PROTEINTECH group

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Material Safety Data Sheet (MSDS)

Protocol for Immunofluorescence with Tissue Culture Cells

1. Culture the cells on small round cover glass.
2. Fix cells with formalin-PBS (10%) for 10 minutes at 4 °C.
3. Transfer the glass into pre-chilled acetone (-20 °C) for exact 5 minutes.
4. Transfer the cover glass into PBS, the cells on cover glass can be stored in PBS-NaN3 for several months at 4°C.
5. Block the cells with BSA-PBS-NaN3.
6. Add 40ul of specific primary antibodies at concentration of 1ug/ml on cover glass and incubate for 1 hour at room temperature in a moist container in dark.
7. Wash the cover glass with 6 cups of PBS-NaN3.
8. Add 40ul of fluorescence-labeled secondary antibody on each cover glass and incubate or 1 hour at room temperature in a moist container in dark.
9. Mount the coverslip in 15% glycerol or a small volume of hydromount on a slide.
10. 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 ddH2O for 5 minutes each.
3. Place slides in vessel filled with antigen retrieval buffer and microwave on middle for 2x2 minutes (700 watt oven), allow retrieval solution to cool at room temperature.
4. Rinse 2 x 5 minutes with TBS.
5. Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in methanol for 30 minutes.
6. Rinse 2 x 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°C overnight,
10. Rinse 2 x 5 minutes with TBST.
11. Apply secondary biotinylated antibody made up in TBS for 1 hour at room temperature.
12. Rinse 2 x 5 minutes with TBS.
13. Apply streptavidin peroxidase for 15 minutes or 30 minutes at room temperature.
14. Rinse 2 x 5 minutes with TBS.
15. Develop with chromogen (DAB) at room temperature, watching under microscope.
16. Rinse in running tap water for 5 minutes.
17. Counterstain in mayor's hematoxylin bath for 30-60 seconds.
18. Wash in water bath 7-8 times, then tap water for 3 minutes.
19. Dehydrate through 60%, 80%, 95% and 100% alcohol for 5 minutes each.
20. Transfer to xylene for 5 minutes. Air for 30 minutes.
21. Mounting.

Protocol for Immunoblotting and Immunoprecipitation:

Preparation of Cell Lysate:

Add RIPA buffer to cells (100ul to a 35 mm dish, 200ul to a 60 mm dish, 500ul 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°C for 5 minutes to remove pellet. Move the supernatant to a fresh tube. Final concentration of cell lysate will be 2-3ug/ul. Add 5 x SDS stop buffer to the lysate to a 1xSDS final concentration.

Immunoblotting:

1. After boiling for 10 minutes, 50-80ul sample will be loaded to SDS PAGE. For most proteins, **100-200ug 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. Cut a piece of membrane to the size of the gel. **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 1mA per cm2.
4. Incubate the membrane in blocking buffer for 1 hour at room temperature or overnight at 4°C. Wash membrane 3x5min with washing buffer.
5. Dilute primary antibody with blocking buffer. Incubate the membrane for 1 hour at room temperature or overnight at 4°C. Wash membrane 3x5min with washing buffer.
6. Dilute secondary antibody with blocking buffer. Incubate the membrane for 1 hour at room temperature. Wash membrane 3x5min with washing buffer.
7. Wash membrane 3x10min with washing buffer.
8. Develop color with ECL.

Immunoprecipitation:

1. Prepare cell lysates or other protein samples. Cellular proteins or other protein samples should be labeled either metabolically or by iodination or biotination. For cell lysate, a concentration of 1-2ug/ul protein is optimum.
2. To 500 ug-1mg cell lysate, add 50ul of 50% slurry of protein A-sepharose beads and incubate the mix at 4°C for 15 minutes. Centrifuge at 10000g for 5 minutes at 4 °C. Collect the supernatant.
3. To the supernatant add 5ug of rabbit polyclonal antibodies against the specific antigen. Gently rock the reaction mixture for 1 hour at 4 °C.
4. Then add 50ul of 50% slurry of protein A-Sepharose beads to the reaction mixture and gently rock the sample at 4 °C for 30 minutes.
5. Spin the sample at 10000 g at 4°C for 3 minutes to pellet the beads.
6. Wash the beads twice with washing buffer A (10mM Tris-HCl, pH 8, 500mM NaCl, 0.5% NP-40 and 0.05% SDS), once with washing buffer B (10mM Tris-HCl, pH 8, 150mM NaCl, 0.5% NP-40, 0.05% SDS and 0.5% Dideoxycholate), once with washing buffer C (10mM Tris-HCl, pH 8, 0.05% SDS).
7. Then add 100ul of 1.25 x SDS loading buffer and boil the sample for 10 minutes before loading to SDS PAGE.
8. Detect the immunoprecipiated sample on SDS-PAGE either by direct radioautography or by further blotting biotin, depending on the initial labeling method.

Buffers

10 x PBS	2000ml
H2O	1000ml
KCl	4g
KH2PO4	4g
Na2HPO4. H2O	22.9g
NaCl	160.0g
ddH2O up to 2 liter	Filter and Autoclave

RIPA buffer	1000ml
150mM sodium chloride	8.76g
1.0% NP-40 or Triton X-100	100ml
0.5% sodium deoxycholate	5g
0.1% SDS	1g
50mM Tris, pH 8.0	6.057g
EDTA	5mM
EGTA	1mM
Na Fluoride	5-10mM
Na Orthovanadate	1mM

Transfer buffer	1000ml
48mM Tris base	5.81g
39mM Glycine	2.93g
0.037% SDS	0.375g
20% MeOH	200ml

Washing buffer TBST	pH7.4
10mM Tris-HCL	
100mM NaCl	
0.2% Tween-20	

Blocking buffer	
Non-fat Milk	5g
Washing buffer TBST pH7.4	100ml

PBS-NaN3	1000ml
Na-Azide	2g
1xPD	1000ml

BSA-PBS-NaN3	1000ml
BSA	5g
1 x PD-Azide	1000ml

TBS	1000ml
Tris base	3g
NaCl	8g
ddH2O	1000ml
Adjust pH to 7.6 with HCL	

TBST	1000ml
TBS	1000ml
Triton X-100	250ul

Antigen retrieval	500ml
0.1 M Sodium Citrate	41ml
0.1 M Citric Acid	9ml
ddH2O	450ml
PH	6.0

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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=27mg/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 re- use.
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°C.

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
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°C
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.

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