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Minute™ Plasma Membrane/Protein Isolation and Cell Fractionation Kit

Catalog number: SM-005

Description

Free of detergents and EDTA, the Minute™ kit is a next-generation solution for native plasma membrane (PM) isolation and cell fractionation. It offers exceptional convenience and consistency by eliminating the variability often seen with traditional methods such as homogenization, density gradient centrifugation, and two-phase partitioning.

How it works: Cells/tissues are first sensitized by buffer A before passing through the proprietary filter in a zigzag manner when high-speed centrifugal force is applied, resulting in a cell lysate containing ruptured cell membranes and intact nuclei. As a result, the nuclear contaminations are virtually eliminated. PM is further separated from the cell lysate (a mixture of crude membranes, intact nuclei, cytosol proteins and organelles) by subsequent differential and density centrifugation with a regular tabletop microcentrifuge. 5 distinct cell fractions (total membrane, PM, cytosol, nucleus and organelles) can be obtained at the completion of the protocol. The procedure can be completed in less than 45 minutes.

Applications

The kit is designed to rapidly isolate native membrane proteins from cultured cells or tissues for applications such as SDS-PAGE, immunoblottings, ELISA, IP, membrane protein structure analysis, 2-D gels, enzyme activity assays and other applications. This kit provides the most rapid method currently available for preparation of native membrane proteins.

Kit components (50 preps):

1. 25 ml buffer A
2. 10 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. 2 plastic rods
6. Tissue dissociation beads

Kit components (4 preps):

1. 2.0 ml buffer A
2. 1.0 ml buffer B
3. 4 protein extraction filter cartridges
4. 4 collection tubes with cap
5. 1 plastic rods
6. Tissue dissociation beads

Storage: Store Buffer A and Buffer B at -20ºC upon arrival.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge

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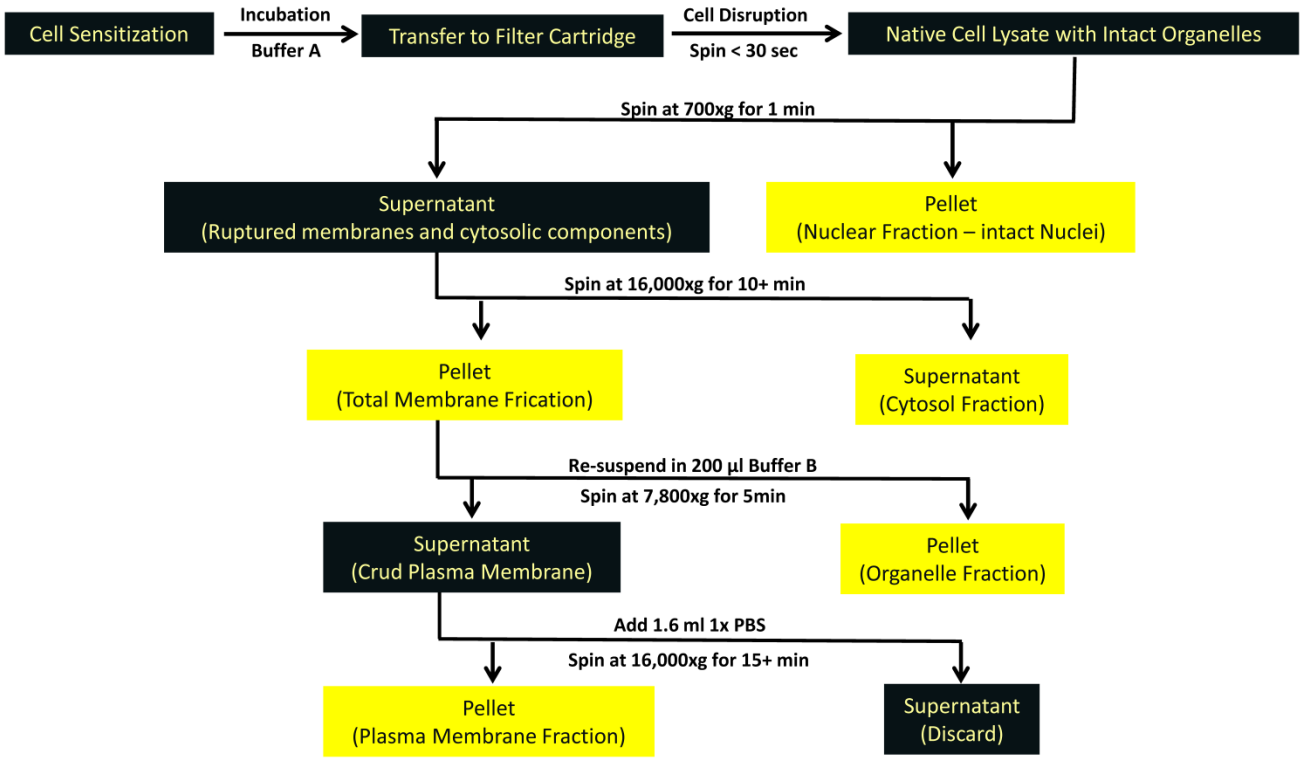
Important Information:

1. Read the entire procedures carefully. Thaw buffer A and buffer B completely, invert the bottles a few times and place them on ice. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. All centrifugation steps should be performed at 4ºC in a cold room or in a refrigerated mirocentrifuge.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. The use of protease inhibitor cocktails is optional.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

The Workflow



Membrane Protein Isolation Procedures:

A. Isolation of Total Membrane Proteins

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1. Place the filter cartridges in collection tubs and incubate on ice.

2. **For cultured cells**:

- Collect 1-50 X 10^6 cells by low-speed centrifugation (500-600 X g 5 min). For adherent cells, please see tech note below for cell harvesting.
  Note: For isolation of plasma membrane proteins from cultured cells (see below) it's recommended to use 20-50 X 10^6 cells.

- Wash cells once with cold PBS (500-600 X g 5 min). Remove supernatant completely and resuspend the pellet in buffer A (200 µl for a starting cell number less than 5 million and 500 µl for a starting cell number greater than 5 million). Incubate the cell suspension on ice for 5-10 min. Vortex the tube vigorously for 10-30 seconds. Immediately transfer the cell suspension to the filter cartridge. Go to step 3.

For tissue samples:

- Place a piece of fresh tissue (10-30 mg) or frozen tissue (20-30 mg) in a filter cartridge. Add 200 µl buffer A to the filter cartridge and grind the tissue with a plastic rod for one min by pushing the tissue against the surface of the filter repeatedly with twisting force
  Note: if you are working with muscles, it is recommended to add 100-120 mg tissue dissociation beads to the filter prior to grinding.

- Add 300 µl buffer A to the same filter cartridge, mix by pipette up and down a few times and incubate on ice with the cap open for 5 min. Go to step 3.

Note: The presence of a small amount of un-homogenized tissue will not affect the quality of the sample. The plastic rod is reusable. For cleaning, wipe it with 75% alcohol or rinse it with distilled water.

3. Cap the filter cartridge and centrifuge at 16,000 X g for 30 seconds (it is recommended to use a tabletop centrifuge that can reach maximum speed in less than 10 seconds).

Optional: For cultured cells, it is recommended to resuspend the pellet in buffer A, and re-pass through the same filter by spinning at 16,000 X g for 30 seconds. The yield may increase by 20-30%.

4. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds.

****The following procedures separate total cellular components into four fractions: nuclei, cytosol, organelles and plasma membrane.***

5. Centrifuge at 700 X g for one min (the pellet contains intact nuclei). Transfer the supernatant to a fresh 1.5 ml microcentrifuge tube and centrifuged at 4ºC for 10-30 min at 16,000 X g (longer centrifugation will increase yield). Remove the supernatant (this is the cytosol fraction) and save the pellet (this is the total membrane protein fraction including organelles and plasma membranes). Store the pellet at -70ºC or dissolve it in detergent-containing buffers of your choice. The yield is typically 10-500 µg/sample. You may stop here if isolation of plasma membrane proteins is not needed. Continue to step 6 for plasma membrane protein

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isolation. Don't freeze total membrane protein fraction if further isolation of plasma membrane proteins is desired.

B. Isolation of Plasma Membrane Proteins:

6. Resuspend the total membrane protein fraction from step 5 in 200 µl buffer B by repeatedly pipetting up and down or vortexing. Centrifuge at 7,800 X g for 5 min at 4ºC (Note: if final plasma membrane prep is contaminated by organelle membranes, increase centrifugation time up to 20 min can improve the purity). The pellet contains organelle membrane proteins.

7. Carefully transfer the supernatant to a fresh 2.0 ml microcentrifuge tube and add 1.6 ml cold PBS. Mix by inverting the tube a few times. Centrifuge at 16,000 X g for 30 min (longer centrifugation will improve yield). Discard the supernatant and save the pellet (isolated plasma membrane). Typically, 10-300 µg plasma membrane proteins can be obtained. Pellet of plasma membrane can be dissolved in 20-200 µl detergent containing buffers of your choice depending upon specific downstream applications. Reagents in following table are recommended for solubilization of the pellet. For isoelectric focusing (First dimension of 2D gel) we recommend 7M urea/2M thio-urea/2% Chaps and 20 mM DTT (add DTT to above mix prior to use).

Tech notes:

1. Harvest adherent cells by trysinization and wash the cells as in step 2. If trysinization is not feasible, the cells can be harvested by a cell scrapper. Scrapped cells should be pipetted up and down repeatedly to contain well-separated cells. Sometimes, it is difficult to obtain accurate cell count with adherent cells. In this case, collect and wash harvested cells by low-speed centrifugation (600 X g 5 min) in a 1.5 ml microfuge tube and compare the wet cell pellet volume to that of 1.5 ml tube containing 40µl water. A wet cell pellet of about 40µl should be used as starting material for plasma membrane isolation.
2. Buffer A may cause cell lysis for certain cell types, such as Jurkat cells, and clog the filter cartridge. This can be resolved by diluting buffer A 1:1 with 1 X PBS.
3. If Western blotting is used for determining plasma membrane enrichment, a total cell lysate must be included as a positive control with equal protein loading. We recommend anti-Na/K-ATPase antibody (Cat# INSM005AB) as a marker of plasma membrane, and Minute Total Protein Extraction Kit (Cat# SD-001/ SN-002) for unbiased.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis

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About Evaluation of Isolated PM Proteins

Many researchers use Western blotting to access the purity of isolated membrane proteins. Some commonly used "cytosolic markers" are not exclusively cytosolic. For example, actin (1), GAPDH (2) and tubulin (3) are mainly cytosolic but they are also associated with plasma membranes. It's not surprising to detect weak signals of these marker proteins in PM preps in certain cell and tissue types. For more info please refer to following publications:

1. Gruenstein E., et al. (1975). Actin associated with membranes from 3T3 mouse fibroblast and Hela cells. Journal of cell Biology. 64:223-234.
2. Terrasse R., et al. (2012). Human and pneumococcal cell surface glyceraldehydes-3-phosphate dehydrogenase (GAPDH) proteins are both ligands of human C1q protein. J. Biol. Chem. 287:42620-42633.
3. Wolff J. (2009). Plasma membrane tubulin. Biochemica et Biophysica Acta. (BBA)-Biomembranes 1788:1415-1433.

Troubleshooting

Problem	Solution
Low protein yield	Increase starting cell numbers Increase incubation time to 10 min (step 2)
Low protein activity	Keep lysate cold/add protease inhibitors
Retention of cell lysate in protein filter cartridge after 30 seconds of centrifugation	Reduce amount of starting material or increase centrifugation time to 2 min
Contamination of PM by cytosolic proteins	Wash PM pellet with 0.5 ml cold PBS containing 0.3 M NaCl, Ph. 9.5

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Minute™ Denaturing Protein Solubilization Reagent

Cat. No. WA-009

Description

This reagent is formulated for dissolving protein pellets using protein kits from Invent Biotechnologies (Cat# Nos. SM-005, SM-005-P, CP-011, NE-013, YM-017, MM-018 and AF-023) and other companies. Solubilized proteins are denatured due to the presence of an anionic detergent (SDS). Protein dissolved in this reagent is mainly used for SDS-PAGE electrophoresis and Western blotting. In some cases, the solubilized protein can also be used for immunoprecipitation and trypsin digestion if SDS is removed using SDS-Remover (Cat. No. WA-012, Invent Biotechnologies).

Use and Procedures

1. Add proper amount of reagent to protein pellet derived from above kits or other methods. The volume used depends upon the size of the pellet and protein concentration required for downstream experiment. Usually 50-200 µl is used/sample.
2. Resuspend the protein pellet in the reagent by pipetting up and down repeatedly (try to avoid bubbles). BCA assay is recommended for determination of protein concentration.
3. Solubilized protein should be mixed with loading buffer prior to loading the gel. Usually, 10-50ug protein/lane is used for a standard Western blot.

Package: 10 ml (For Research Use Only)

Shipping and Storage: Ship at ambient temperature and stored at RT.

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Minute™ Total Protein Extraction Kit for Animal Cultured Cells and Tissues

Cat #: SD-001/SN-002

Description

The Minute™ kit is a next-generation, spin column–based system designed for rapid and efficient total protein extraction from animal cultured cells and tissues. Unlike conventional solution-based methods, such as RIPA buffer, which often result in protein loss and altered profiles, this patented technology preserves protein integrity and yields reproducible results.

With enhanced lysis buffers and a streamlined protocol, proteins can be extracted in just 1–5 minutes at concentrations ranging from 1–8 mg/ml. The system supports both denaturing and native conditions, allowing users to choose based on downstream applications. Minimal extraction volume (as low as 20 µl) makes it ideal when starting material is limited.

Application

The Minute™ Total Protein Extraction Kit enables rapid and efficient isolation of total proteins from both invertebrate and vertebrate cultured cells and tissues. It is optimized for common downstream applications including SDS-PAGE, Western blotting, immunoprecipitation (IP), ELISA, and enzyme activity assays.

This kit offers one of the fastest protocols available for total protein preparation and can be readily adapted for high-throughput workflows. The extracted proteins are also suitable as starting material for small-scale purification using column chromatography.

Kit components(50 Preps):

1. 25 ml denaturing cell lysis buffer (SD-001)
2. 25 ml Native cell lysis buffer (SN-002)
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. Plastic rods (2)

Kit components(4 Preps):

1. 2.0 ml denaturing cell lysis buffer (SD-001)
2. 2.0 ml Native cell lysis buffer (SN-002)
3. 4 protein extraction filter cartridges
4. 4 collection tubes with cap
5. Plastic rods (1)

NOTE: **Cell lysis buffers listed above do not contain any reducing agents and primary amine

Shipping: This kit is shipped at ambient temperature
Storage: Store the kit at room temperature

Additional Guidelines for Optimal Use

The Minute™ Total Protein Extraction Kits are engineered for rapid and efficient protein extraction. The inclusion of protease inhibitors in the lysis buffer is optional; however, their use is recommended if the extracted proteins will be stored or if downstream applications are time-consuming, to prevent proteolytic degradation.

For accurate protein quantification, the BCA Protein Assay Kit (Pierce) is recommended.

For phosphorylation studies, phosphatase inhibitors—such as PhosSTOP (Roche)—should be added to the lysis buffer prior to use to preserve phosphorylation states.

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If precipitate is found in Denaturing Buffer at lower temperature, incubate at >37^{0}C until the precipitate is completely dissolved.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge
BCA Protein Assay Kit (Pierce, Cat #. 23227)

Protocols:

Total Protein Extraction for Cultured Cells

1. Place a filter cartridge into a collection tube and keep it on ice.
2. Harvest cells by low-speed centrifugation. Wash the cell pellet once with cold PBS in a 1.5 ml microcentrifuge tube.
3. Pellet cells by centrifugation at 500-600 X g for 3 min. Discard the supernatant.
4. Add 20-500 µl of lysis buffer (SD-001 or SN-002) to the cell pellet. Vortex for 10–20 seconds to fully lyse the cells. ***Refer to Tables 1–4 for recommended lysis buffer-to-cell ratios.***
5. Transfer the lysate to the filter cartridge in the collection tube. Centrifuge at 14,000–16,000 × *g* for 30 seconds using a tabletop centrifuge.
6. Discard the filter cartridge. The filtrate in the collection tube contains the extracted total protein, ready for downstream applications.

Total Protein Extraction for Animal Tissues

1. Place 1–20 mg of fresh or frozen tissue into the filter cartridge positioned in the collection tube. Using a plastic rod, grind the tissue with a twisting motion 20–30 times.
2. Add 50–200 µl of lysis buffer (SD-001 for denaturing or SN-002 for native extraction) directly into the filter cartridge. Continue grinding for another 10–20 strokes to complete tissue homogenization.
   Note: The plastic rod is reusable. Rinse thoroughly with distilled water and dry with a clean paper towel.
3. Cap the filter cartridge and centrifuge at maximum speed (14,000–16,000 X *g*) for 30 seconds to 1 minute using a tabletop centrifuge.
4. Discard the filter cartridge. The filtrate in the collection tube contains the extracted total protein, ready for downstream applications.

High-Throughput Applications

The filter cartridges, in combination with lysis buffers (SD-001 or SN-002), are well-suited for high-throughput applications, enabling rapid and consistent extraction of total protein from multiple animal tissue samples or cultured cells. For optimal performance in high-throughput workflows, a sample mixer or vortexer equipped with an attachment capable of holding multiple 2 mL Eppendorf tubes, and operating at a minimum speed of 2,500 rpm, is required.

1. Place 5–30 mg of fresh or frozen tissue into the filter cartridges seated in collection tubes.
2. Add 100–300 µl of lysis buffer (SD-001 for denaturing or SN-002 for native conditions) into each filter cartridge. Cap the filters securely and placed in a multisampling adaptor.
3. Vortex the sealed filter cartridges in their collection tubes at 2,000–2,500 rpm for 3–5 minutes at room temperature.

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4. After vortexing, transfer all samples—along with the mixing attachment—into a compatible rotor adapter (e.g., a 96-well plate adapter) and centrifuge at maximum speed for 1–2 minutes. Alternatively, remove the filter cartridges along with the collection tubes and centrifuge them in a tabletop centrifuge at top speed for 30 seconds to 1 minute.
5. The filtrate collected in the tube is the total protein extract, ready for downstream applications. Protein yield typically ranges from 1–5 mg/ml.

Note: a). For cultured cell samples, please refer to Tables 1–4 for buffer volumes and handling. Vortexing (Step 3) can generally be omitted.
b). While increased vortex speed and duration may enhance protein yield, do not exceed 28,000 rpm or vortex for longer than 10 minutes, as this may compromise protein integrity.

Recommendations for Protein Extraction from Cultured Cells

-Total Protein Extraction Using Denaturing Lysis Buffer (SD-001)

A. **For Cells in Suspension**. Harvest cells by centrifugation at 500-600 X g for 3 min. Wash cells with 1 X cold PBS and estimate wet cell pellet volume.

Table 1. Lysis Buffer Volume Recommended for Different Packed Cell Volumes*

Packed cell volume (µl)	lysis buffer (µl)	Equivalent cell # X 10^6
3	20	0.3
5	50	0.5
10	100	1
20	200	2

B. **For Adherent cells.** Wash cells with 1 X PBS and lyse cells in the culture container by pipetting up and down for 10-20 times.

Table 2. Amounts of lysis buffer recommended for different number of adherent cells

Containers	Approximate Cell#	Lysis buffer(µl)
24-well plate	0.1-0.2 Million	50
6-well plate	0.6-0.8 Million	200
25 cm^2 flask	1.5-2 Million	500

Note: this is a general reference, the actual amount of lysis buffer can be more or less

-Total Protein Extraction Using Native Lysis buffer (SN-002)

A. For Cells in Suspension. Harvest cells by centrifugation at 500-600 X g for 3 min. Wash cells with 1 X PBS and estimate wet volume.

Table 3. Lysis buffer volume recommended for different packed cell volumes*

Packed cell volume (µl)	lysis buffer (µl)	Equivalent cell # x 10^6
3	25	0.3
5	50	0.5
10	100	1

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20	200	2

B. For Adherent cells. Wash cells with 1 X PBS and lyse cells in the culture container by pipetting up and down for 10-20 times

Table 4. Amounts of Lysis Buffer recommended for Different Amount of Adherent Cells

Containers	Approximate Cell#	Lysis buffer((µl)
24-well plate	0.1-0.2 Million	50
6-well plate	0.6-0.8 Million	250
25 cm^2 flask	1.5-2 Million	500

Note: this is a general reference, the actual amount of lysis buffer can be more or less

Tech Notes:

1. The protocols can be performed at room temperature.
2. The filter cartridge may accommodate more starting material than recommended, as long as it does not become clogged after centrifugation.
3. The cells can be washed with 0.5 to 1 ml cold PBS 1-2 times.
4. Denaturing buffer usually yields more protein than native buffer because of high cell lysis efficiency.
5. If very small amount of tissue is used (<5 mg), incubation at room temperature for 5 min after plastic rod homogenization may increase the yield.
6. Extracted protein may be stored at -80°C for long term storage.

Troubleshooting

Problem	Solution
The lysate is too viscous to pipette with a 200-1000 µl pipette tip	Pour the lysate into protein extraction filter cartridge
Retention of cell lysate in filter cartridge after 30 seconds of centrifugation	Decrease amounts of starting cells/tissues or increase amount of lysis buffer
Low protein concentration Low protein band intensity at high molecular weight range (100-300 KDa)	Increase amounts of cells/tissues or decrease amount of cell lysis buffer; Increase amount of lysis buffer and make sure cells/tissues are completely lysed.

Invent ® Na+/ K+ ATPase α1
Recombinant Rabbit Monoclonal Antibody

Cat. No. IN-SM005AB

Description:

Na+/ K+ ATPase exists in cell membrane and is an active transport enzyme that can exchange sodium and potassium ions. It was discovered by J.C. Skou in 1957. With a few exceptions, such as the red blood cell membrane of cats and dogs, it exists in all animal cell membranes. It has also been reported in bacteria, but instances are rare, and its presence in plants has not been confirmed. It is only active when Na+ and K+ are present simultaneously. During the decomposition of ATP, the enzyme molecules undergo allosteric (conformational change), which makes the bound Na+ and K+ transfer from inside the cell membrane to outside the cell membrane, or from outside the cell membrane to inside the cell membrane. It can be used as a marker for cell membranes (plasma membrane).

Application:

The antibody can be applied to WB, IHC, ICC/IF, Flow Cytometry

Important Product Information:

Size： 20ul / 100ul

Species Reactivity： Human, Mouse, Rat

Host/Isotype： Rabbit / IgG

Concentration： 1 mg/ml

Molecular Wt: Predicted band size: 113 kDa

Shipping:

This antibody is shipped at 4°C.

Storage:

Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Additional Materials Required：

centrifuge
Antibody diluent

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Protocols:

1. Place the antibody tube into the centrifuge and increase the speed to 4000rpm to allow the liquid to the bottom of the tube.
2. Refer to Table 1 to dilute the antibody proportionally for different applications.

Table 1. Tested Dilution for Different Applications

Applications	Tested Dilution
Western Blot (WB)	1:1,000-1:5,000
Immunohistochemistry (IHC)	1:50-1:200
Immunocytochemistry (ICC/IF)	1:50-1:200
Flow Cytometry (Flow)	1:50-1:100

Tech notes:

Membrane proteins tend to form polymers at high temperatures, and it is recommended not to exceed **70 ℃** when preparing samples with WB.

For Research Use Only. Not for use in diagnostic procedures.