ExKine™ Total Membrane Protein Extraction Kit

Cat #: KTP3004 Size: 50 T/200 T

	Total Membrane Protein Extraction Kit		
REF	**Cat #:** KTP3004	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells		
	Storage: Stored at -20℃ for 12 months		

Assay Principle

The preparation of an extract from membrane is often the first step in studying membrane proteins and their interactions. The resulting preparation can be used directly in Western blotting, Dot blot, Immunoprecipitation, or as a starting point for the purification of specific proteins. ExKine™ Total Membrane Protein Extraction Kit is a simple, rapid and reproducible method to prepare cellular protein fractions highly enriched in membrane and hydrophobic proteins, based on temperature-dependent phase separation. The sample is first mixed and homogenized in the membrane protein extraction buffers. After a brief incubation at 37℃, the sample is centrifuged, producing an upper aqueous phase and a lower detergent-rich phase. Proteins that were anchored to the membrane or proteins containing one or two transmembrane domains are efficiently partitioned to the detergent-rich phase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	200 T	
Extraction Buffer (2×)	25 mL	100 mL	4℃
Wash Buffer (2×)	15 mL	60 mL	4℃
Protease Inhibitor (100×)	0.8 mL	3.2 mL	-20℃

Materials Required but Not Supplied

- Vortexer, centrifuge tube
- Cell scraper
- Precision Pipettes, Disposable Pipette Tips
- Phosphate buffered saline (PBS), Deionized Water
- Dounce homogenizer(for Tissue Samples)

Reagent Preparation

Working Extraction Buffer (1×): Before use, dilute with deionized water to 1×Extracion Buffer, immediately add 10 μL Protease Inhibitor (100×) to 1 mL 1×Extracion Buffer, place on ice; store at 4℃.

Working Wash Buffer (1×): Before use, dilute with deionized water to 1×Wash Buffer, immediately add 10 μL Protease Inhibitor (100×) to 1 mL 1×Wash Buffer, place on ice; store at 4℃.

Protease Inhibitor (100×): Ready to use as supplied. Place on ice before use; store at -20℃. The remaining working solution can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

Assay Procedure

Note: Perform all steps at 2-8℃. Use precooled buffers and equipment. Ensure all the solutions are defrosted and homogeneous.

I Cell Culture Preparation

1. For adherent cells, harvest 1-3×10^7 cells with cell scrapers and then centrifuge at 500 g for 5 min. For suspension cells, harvest by centrifuging at 500 g for 5 min.
2. Wash cells by suspending the cell pellet with cold PBS. Centrifuge at 500 g for 2-3 min and discard the PBS.
3. Add 1 mL cold 1×Working Extracion Buffer to the cell pellet, homogenize the cells 30-50 times with a homogenizer, and continue to procedure III.

II Tissue Preparation

1. Cut 50-100 mg of tissue into small pieces and put them in a centrifuge tube.
2. Wash the tissue with cold PBS, centrifuge at 500 g for 5 min, and discard the supernatant.
3. Add 1 mL cold 1×Working Extracion Buffer to gently resuspend the tissue.
4. Use a homogenizer or tissue grinder to homogenize the tissue until more than 90% of the cells are destroyed and the nucleus is visible under the microscope, continue to procedure III.

III Separation of hydrophobic and hydrophilic proteins

1. Incubate the cell suspension on ice for 10 min.

Note: 50-100 μL aliquot of the total protein lysate may be saved for further analysis.

2. Centrifuge at 10,000 g for 5 min at 4℃.
3. Transfer the supernatant to a new microcentrifuge tube and discard the pellet. The supernatant was incubated at 37℃ for 5 min until it became cloudy, and during the incubation period, the tube was inverted upside and down to mix thoroughly.
4. Centrifuge at 3,000 g for 3 min at room temperature.

Note: Ensure the centrifuge temperature is higher than 20℃. Do not return the tube to ice after centrifugation. Pay attention to observe the liquid stratification after centrifugation, the solution after centrifugation must have obvious stratification, otherwise repeat this step. The upper liquid is a hydrophilic phase (cytoplasmic protein), and the lower layer is a viscous oil. The hydrophobic phase contains hydrophobic proteins (membrane proteins).

5. The lower hydrophobic phase is greatly enriched with hydrophobic and raft associated proteins. Transfer the upper hydrophilic phase containing hydrophilic proteins to a new microcentrifuge tube for further analysis.
6. In order to remove residual hydrophilic proteins from the hydrophobic phase, add 400 μL1×Working Wash Buffer to the hydrophobic phase. Mix gently and incubate the tube on ice for 10 min. Repeat steps 3-5.
7. If no further downstream applications are to be immediately performed, aliquot and quick-freeze the hydrophobic membrane protein fractions on dryice and then store at -80℃.
8. For SDS-PAGE: directly use the separated membrane protein and boil it for electrophoresis. For IP experiments: Before adding antibody, use 1×Working Wash Buffer to dilute the hydrophobin by 10-15 times.

Note: If necessary, the sample can be precipitated by TCA to obtain a more concentrated sample.

Recommended Products

Catalog No.	Product Name

KTP3001	ExKine™ Nuclear and Cytoplasmic Protein Extraction Kit
KTP3002	ExKine™ Nuclear Protein Extraction Kit
KTP3003	ExKine™ Cytoplasmic Protein Extraction Kit
KTP3005	ExKine™ Membrane and Cytoplasmic Protein Extraction kit
KTP3006	ExKine™ Total Protein Extraction Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.