ExKine™ Membrane and Cytoplasmic Protein Extraction Kit

Cat #: KTP3005 Size: 50 T/200 T

	Membrane and Cytoplasmic Protein Extraction Kit		
REF	**Cat #:** KTP3005	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells		
	Storage: Stored at -20℃ for 12 months		

Assay Principle

ExKine™ Membrane and Cytoplasmic Protein Extraction Kit enable stepwise separation and preparation of non-denatured, active cytoplasmic and membrane protein from mammalian cultured cells or tissues, which can be used directly in a variety of proteomics application. The cells are first broken by homogenization to allow the release of cytosolic and membrane proteins, after which membrane proteins are obtained by high speed centrifugation. Extraction efficiencies and yields will vary depending on cell type as well as the number of times the integral membrane protein spans the lipid bilayer.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	200 T	
Cytoplasmic Extraction Buffer (CEB) (5×)	10 mL	40 mL	4℃
Membrane Extraction Buffer (MEB)	10 mL	40 mL	4℃
Protease Inhibitor (100×)	0.6 mL	2.4 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 Cytoplasmic Extraction Buffer (Working CEB) (1×): Before use, prepare 1×CEB with deionized water, immediately add 10 µL Protease Inhibitor (100×) to 1 mL 1×CEB, place on ice; store at 4℃.

Working Membrane Extraction Buffer (Working MEB): Before use, add 10 µL Protease Inhibitor (100×) to 1 mL MEB, 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.

Note: Use a pipette to carefully remove and discard the PBS, leaving the cell pellet as dry as possible.

3. Add 1 mL cold 1×Working CEB to the cell pellet. Proceed to procedure III, vortex the tube vigorously at the highest setting for 15 s to completely suspend the cells.
4. Homogenize the cells 30-50 times using a homogenizer on ice until more than 90% of the cells are broken and the nucleus is visible under the microscope. Proceed to procedure III.

II Tissue Preparation

1. Cut 50-100 mg of tissue into small pieces and place in a centrifuge tube.
2. Wash tissue with PBS. Centrifuge tissue at 500 g for 5 min and discard the PBS.

Note: Use a pipette to carefully remove and discard the PBS, leaving the sample as dry as possible.

3. Resuspend the tissue gently in 1 mL cold 1×Working CEB.
4. Homogenize tissue using a homogenizer or a tissue grinder until more than 90% of the cells are broken and nuclei are visualized under the microscope. Proceed to procedure III.

III Extraction of cytoplasmic protein and membrane protein

1. Centrifuge at 800 g for 5 min at 4℃. Then transfer the supernatant to a clean cold centrifuge tube. The pellet contains nuclei and unruptured cells.
2. Centrifuge at 16,000 g for 45 min at 4℃.
3. Transfer the supernatant to a clean cold centrifuge tube (cytoplasmic extract). The precipitate contains membrane proteins. The cytoplasmic fraction stored on ice should be used immediately or the sample should be aliquoted and stored at -80℃.

Optional step: In order to remove residual cytoplasmic proteins from the membrane, wash the pellet with other ice-cold Working CEB or PBS. Then repeat steps 2 and 3.

4. Suspend the precipitate in 200 µL of cold Working MEB, place it on ice, and vortex for 15 s every 10 min for 30 min. Avoid foam formation.
5. Centrifuge at 16,000 g for 5 min at 4℃.
6. Dispense the supernatant (membrane extract) into a cold centrifuge tube, and take out a small aliquot for protein quantitative detection. Store the other centrifuge tubes containing the membrane extract at -80℃. Avoid repeated freezing and thawing.

Precautions

Problem	Possible Cause	Solution
Low protein concentration of membrane fraction	Cell was not lysed	Increase incubation time following addition of CEB or strokes of homogenization
	Incomplete membrane protein isolation	Increase time of centrifugation following addition of CEB
	Incorrect volumes or mistake made in addition	Make buffers carefully

	of buffers used for lysis or extraction	
Proteins not compartmentalized	Incomplete removal of cytoplasmic extract	Carefully remove all cytoplasmic Proteins not extract before membrane lysis
		Rinse membrane pellet with additional Working CEB or PBS
Low overall protein yeild	Not enough cells/tissue	Increase cell number or amount of starting tissue (mg)

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
KTP3004	ExKine™ Total Membrane 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.