ExKine™ Cytoplasmic Protein Extraction Kit

Cat #: KTP3003 Size: 50 T/200 T

	Cytoplasmic Protein Extraction Kit		
REF	**Cat #:** KTP3003	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 nuclei is often the first step in studying nuclear proteins and their interactions. The resulting preparation can be used directly in Western blotting, Electrophoresis Mobility Shift Assay (EMSA), footprinting analysis, transcription assays, or as a starting point for the purification of regulatory proteins. ExKine™ Cytoplasmic Protein Extraction Kit enable stepwise separation and preparation of crude cytoplasmic extracts from mammalian cultured cells or tissues. This Kit is based on allowing cells to swell with hypotonic buffer. And then the cells are disrupted, the cytoplasmic fraction is removed. Non-denatured, active proteins are purified in less than two hours.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	200 T	
Cytoplasmic Solution A (CESA)	10 mL	40 mL	4℃
Cytoplasmic Solution B (CESB)	0.5 mL	2 mL	4℃
DTT (500×)	25 μL	100 μL	-20℃
Protease Inhibitor (100×)	0.15 mL	0.5 mL	-20℃

Materials Required but Not Supplied

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

Reagent Preparation

Working Cytoplasmic Solution A (Working CESA): Before use, add 10 μL Protease Inhibitor (100×) and 2 μL DTT (500×) to 1 mL CESA, place on ice; store at 4℃.

Cytoplasmic Solution B (CESB): Ready to use as supplied. Place on ice before use; store at 4℃.

DTT (500×): 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.

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 2×10^6 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 200 μL cold Working CESA to the cell pellet. Proceed to procedure III.

II Tissue Preparation

1. Cut 30-60 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 200 μL cold Working CESA.
4. Homogenize tissue using a Dounce 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 Cytoplasmic Protein Extraction

1. Vortex the tube vigorously on the highest setting for 15 s to fully suspend the cell pellet. Incubate the tube on ice for 15 min to allow cells to swell.
2. Centrifuge the tube at 800 g for 5 min at 4℃. and the supernatant was immediately transferred to a clean centrifuge tube.
3. Add 10 μL cold CESB to th e centrifuge tube and place on ice, vortexed for 15s per 10 min for 30 min. Avoid foam formation.
4. Centrifuge the tube at 16,000 g for 5 min at 4℃.
5. Dispense the supernatant (cytoplasmic extract) into a cold centrifuge tube, and take out a small aliquot for protein quantitative detection. Store the other centrifuge tubes containing cytoplasmic extract at -80℃. Avoid repeated freezing and thawing.

Precautions

Problem	Possible Cause	Solution
Low protein concentration of cytoplasmic fraction	Volume of lysis or extraction buffer does not correspond to correct number of cells	Count cells and use appropriate buffer volumes
	Cells were not lysed	Vortex thoroughly
	Tissues were homogenized in PBS	Homogenize tissues in Working CESA
Proteins not compartmentalized	Incomplete lysis of cells	Increase vortexing time to adequately disperse the cell pellet recommended incubation times
		Increase amount of CESB

Recommended Products

Catalog No.	Product Name
KTP3001	ExKine™ Nuclear and Cytoplasmic Protein Extraction Kit
KTP3002	ExKine™ Nuclear Protein Extraction Kit
KTP3004	ExKine™ Total Membrane 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.