Nuclear and Cytoplasmic Protein Extraction Kit

Cat#: orb1173400 (User manual)

Nuclear and Cytoplasmic Protein Extraction Kit
Cat #: orb1173400
Size: 50 T/200 T
Lot #: Refer to product label
Applicable samples: Animal Tissues, Cells
Storage: Stored at -20℃ for 12 months

Assay Principle

The extraction of nuclear and cytoplasmic proteins can not only be used to study the localization of proteins in cells, but also in many cases. The extraction proteins can be used for the study of transcriptional regulation, such as Western blotting, Electrophoresis Mobility Shift Assay (EMSA), footprinting analysis, transcription assays, or as a starting point for the purification of regulatory proteins. Nuclear and Cytoplasmic Protein Extraction Kit enable stepwise separation and preparation of crude cytoplasmic and nuclear 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, and the nuclear proteins are released from the nuclei by a high salt buffer. 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℃
Nuclear Extraction Solution (NES)	5 mL	20 mL	4℃
DTT (500×)	40 μL	130 μL	-20℃
Protease Inhibitor (100×)	0.2 mL	0.7 mL	-20℃

Materials Required but Not Supplied

·Vortexer, centrifuge tube
·Microscope, 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℃.
Working Nuclear Extraction Solution (Working NES): Before use, add 10 µL Protease Inhibitor (100×) and 2 µL DTT (500×) to 1 mL NES, place on ice, 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 2×106 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 until more than 90% of the cells are broken and nuclei are visualized under the microscope. Proceed to procedure III.

III Cytoplasmic and Nuclear 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. Add 10 µL cold CESB to the tube. Vortex the tube for 10-15 s on the highest setting. Incubate tube on ice for 1-2 min.
3. Centrifuge the tube at 16,000 g for 5 min at 4℃.
4. Immediately transfer the supernatant (cytoplasmic extract) to a clean cold tube. Place this tube on ice until use or at -80℃ for longer storage. The pellet (which contains nuclei) is usually viscous and not very compact.

Optional: In order to remove residual cytoplasmic protein from the nuclei, rinse the pellet with additional cold Working CESA buffer or PBS. And then repeat procedure III in Step 3 and 4 for 1-2 times.
5. Add 100 µL of pre-cooled Working NES to resuspend the pellet. Place the sample on ice and continue vortexing for 15 s every 10 min for 30 min. Avoid foam formation.
6. Centrifuge at 16,000 g for 15 min at 4℃.
7. Dispense the supernatant (nucleoprotein) into a cold centrifuge tube, and take out a small aliquot for protein quantitative detection. Store the other centrifuge tubes containing nucleoprotein at -80℃. Avoid repeated freezing and thawing.

Note: The nuclear proteins extracted according to the protocol are suspended in NES, a high salt buffer. If large volumes of nuclear extract are required in subsequent applications or if problems occur with downstream assays, dialyze the nuclear extract to remove excess salts before use.

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
	Cell pellet was not dispersed	Vortex thoroughly
	Cells were not lysed	Increase amount of CESB
	Tissues were homogenized in PBS	Homogenize tissues in Working CESA
Low protein concentration of nuclear fraction	Cell pellet was not dispersed	Vortex thoroughly
	Incomplete nuclei isolation	Increase time of centrifugation following addition of CESB
	Incorrect volumes or mistake made in addition of buffers used for lysis or extraction	Make buffers carefully
Proteins not compartmentalized	Incomplete lysis of cells	Increase vortexing time to adequately disperse the cell pellet recommended incubation times
		Increase amount of CESB
	Incomplete removal of cytoplasmic extract	Carefully remove all cytoplasmic extract before nuclear lysis
		Rinse nuclei with additional Working CESA buffer or PBS