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ExKine™ Nuclei Extraction Kit

Cat #: KTP4001 Size: 50 T/200 T

	Nuclei Extraction Kit		
REF	**Cat #:** KTP4001	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells		
	Storage: Stored at 4℃ for 12 months		

Assay Principle

The preparation of an extract from nuclei is often the first step in for many cell biology applications, such as in vitro apoptosis, examination of the transcriptional status of cells and a source of nuclear components (chromatin, genomic DNA, histones and nuclear RNA/RNP). The ExKine™ Nuclei Extraction Kit enable rapid isolation of nuclei from mammalian cultured cells and homogenate tissues. The isolated nuclei can be preserved frozen several months in the included storage buffer.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	200 T	
Lysis Buffer (10×)	20 mL	80 mL	4℃
Nuclei Storage Buffer	10 mL	40 mL	4℃

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

Lysis Buffer (1×): Diluting 10-fold Lysis Buffer with sterile, deionized water. Place on ice before use, store at 4℃.
Nuclei Storage Buffer: Ready to use. Place on ice before use, store at 4℃.

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 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 3 mL ice-cold 1× Lysis Buffer to the cell pellet. Vortex cells for 10 s at half maximal speed.
4. Incubate lysed cells for 5 min on ice. Proceed to procedure III.

Note: Examine a few microliters of cell lysate with a microscope to ensure that cells have uniformly lysed and nuclei appear free of cytoplasmic material.

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 with 3 mL 1×Lysis Buffer.
4. Homogenize tissue using a Dounce homogenizer incubate on ice for 15 min. Proceed to procedure III.

III Nuclear Extraction

1. Centrifuge the tube at 500 g for 5 min at 4℃, remove supernatant.

Note: The supernatant contains cytoplasmic components and can be saved for later analysis or use.

2. Resuspend the pellet with 1 mL ice-cold 1×Lysis Buffer. Vortex cells for 10 s at half maximal speed.
3. Centrifuge at 500 g for 5 min at 4℃. Carefully aspirate the clear supernatant and set the nuclei pellet on ice.
4. Loosen nuclear pellet by gently vortexing 5 s. Add 200 μL ice-cold Nuclei Storage Buffer and resuspend nuclei by pipetting up and down (Nuclei will be clumped at first but will disperse with continued pipetting. Pipetting should be steady but should not create air bubbles).

Note: (1) Nuclei should be used immediately or frozen at -80℃ for storage. Nuclei frozen at -80℃ in Nuclei storage buffer are stable for at least several months. (2) The number and purity of the final nuclei can be quickly determined by visual microscopic inspection of the nuclei staining with trypan blue counting solution (It is recommended to dilute trypan blue with Nuclei Storage Buffer to prevent swelling of nuclei). Nuclei will stain blue with a uniform circular or sausage-shaped appearance, whereas cytoplasmic contamination and cell debris will stain light blue with an irregular morphology and will be clearly visible, if present.

Recommended Products

Catalog No.	Product Name
KTP4002	ExKine™ Nuclei Extraction Kit (High Purity)
KTP4003	ExKine™ Mitochondrion Extraction Kit (Cultured Cells)
KTP4004	ExKine™ Mitochondrion Extraction Kit (Tissue)

Disclaimer

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