ExKine™ Nuclei Extraction Kit (High Purity)

Cat #: KTP4002 Size: 20 T/100 T

	Nuclei Extraction Kit (High Purity)		
REF	**Cat #:** KTP4002	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). ExKine™ Nuclei Extraction Kit (High Purity) is designed for the preparation of pure nuclei and fragile nuclei from mammalian cultured cells and solid tissues. Nuclei are collected by ultracentrifugation through a sucrose cushion and are quite clean and free of contaminating membranes and cytoplasmic components. The isolated nuclei can be preserved frozen several months in the included storage buffer.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	20 T	100 T	
Lysis Buffer	20 mL	100 mL	4℃
1.8 M Sucrose Cushion	90 mL	200 mL	4℃
Sucrose Dilution	4 mL	20 mL	4℃
Nuclei Storage Buffer	4 mL	20 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: Ready to use. Place on ice before use, store at 4℃.

1.8 M Sucrose Cushion: Ready to use. Place on ice before use, store at 4℃.

Sucrose Dilution: Ready to use. 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 1 mL ice-cold Lysis Buffer to the cell pellet. Vortex cells for 10 s at half maximal speed.
4. Transfer to an ice-cold Dounce homogenizer and break the cells at 2-8℃ until the nuclei appear free of cytoplasmic tags (usually 15-30 strokes). 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 1 mL Lysis Buffer.
4. Homogenize tissue with a Dounce homogenizer or a tissue grinder for 15-30 strokes at 2-8℃ and Check a few microliters of cells with a phase-contrast microscope to be sure they are uniformly lysed. Proceed to procedure Ⅲ.

III Nuclear Extraction

1. Add 1 mL ice-cold 1.8 M Sucrose Cushion (The volume can be adjusted according to actual needs) to each lysate sample on ice. Mix by gentle pipetting and inversion.
2. For each sample preparation, add 1 mL of ice cold 1.8 M Sucrose Cushion to the bottom of a ultracentrifuge tube on ice.
3. Carefully and slowly layer the 2 mL of lysate solution from step Ⅰ or II on top of the 1.8 M Sucrose Cushion from step Ⅲ. Avoid disturbing the Sucrose Cushion layer.

Note: Nuclei from different cell types or tissues may have different densities and may require different concentrations of sucrose for the sucrose cushion used in this purification step for maximum results. The sucrose concentration can be adjusted using Sucrose Dilution.

4. Carefully place the filled ultracentrifuge tubes into pre-cooled buckets of a Ultracentrifuge and centrifuge the gradient for 45 min at 30,000 g, 4℃.
5. Carefully and completely aspirate the supernatant (cytoplasm and cell debris) and the clear sucrose cushion layers without disturbing the pellet of purified nuclei.

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

6. 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).
7. Store frozen nuclei at -80℃ or in liquid nitrogen.

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
KTP4001	ExKine™ Nuclei Extraction Kit
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.