Nuclear/Cytosol Fractionation Kit

Cat. No. KITG-018 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit can separate nuclear and cytosolic fractions from animal cells. The kit provides a system that maintains nuclear and cytosolic fractions as separate and intact. The optimized reagent formulation and procedure allow rapid isolation of nuclear and cytosolic fractions with minimal cross-contamination. The extracted nuclear and cytosolic fractions retain their biochemical functions and are suitable for downstream analyses such as transcriptional activity, RNA splicing, gel-shift assays, reporter assays, enzyme activity assays, and Western blot. The cytosolic fraction obtained by this kit contains organelles. If a cytosolic fraction (cytosol) without organelles is required, other kits should be selected.
Species	Animal cells
Description	The Nuclear/Cytosol Fractionation Kit is designed to isolate nuclear and cytosolic fractions from animal cells with minimal cross-contamination. The kit uses a two-step extraction procedure: first, cells are lysed in Extraction Buffer A to release the cytosolic fraction; then, the nuclear pellet is washed with PBS and resuspended in Storage Buffer B. The extracted fractions retain their biochemical functions and are suitable for various downstream applications including transcriptional activity analysis, RNA splicing, gel-shift assays, reporter assays, enzyme activity assays, and Western blot.
Purity	Minimal cross-contamination between nuclear and cytosolic fractions.
Applications	Isolation of nuclear and cytosolic fractions from animal cells for downstream analyses

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	including transcriptional activity, RNA splicing, gel-shift assays, reporter assays, enzyme activity assays, and Western blot.
Notes	1. Protease inhibitors can be stored at 2-8°C before opening. After opening, store at -20°C. 2. Protease inhibitors are in solid state at low temperature (2-8°C). Remove from refrigerator and allow to return to room temperature or briefly warm at 37°C in a water bath until liquid state, then centrifuge to the bottom of the tube before opening. 3. Please use the reagents as soon as possible after opening. 4. Pre-experiments are very important. Pre-experiments must be performed before formal experiments. Take a small amount of sample to optimize experimental conditions, and conduct the pre-experiment as carefully as the formal sample to see if the experimental results are feasible and whether the experimental conditions are suitable for your sample. Due to the diversity of biological samples, experimental conditions for different samples usually vary greatly, and different models of the same cell type may require different experimental conditions. To avoid wasting formal samples and reagents, pre-experiments must be performed in advance to optimize experimental conditions. 5. For reagents in screw-cap centrifuge tubes, please briefly centrifuge before opening to spin down liquid on the inner wall of the cap to avoid spillage when opening. 6. All reagents must be pre-cooled during the experiment; all instruments must be pre-cooled in a -20°C refrigerator. The entire process must keep samples at low temperature. 7. If precipitate appears in the protease inhibitor solution during storage, it does not affect use. Dissolve and use normally. 8. Other protease inhibitor single products can be added according to experimental needs.
Storage	Store at 2-8°C. Protease inhibitors: before opening, store at 2-8°C; after opening, store at -20°C.
Concentration	50T / 100T
Reaction conditions	Instruments Required: 1. Centrifuge 2. Shaker 3. Vortex mixer 4. Pipette 5.

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	Refrigerator 6. Ice box Reagents Required: 1. PBS buffer 2. Protein quantification kit Consumables Required: 1. Centrifuge tubes 2. Pipette tips 3. Disposable gloves Procedure: 1. Take 5-10×10^6 cells, centrifuge at 4°C, 500×g for 3 min. Carefully aspirate the culture medium, remove as much as possible, and collect the cells. 2. Wash the cells twice with cold PBS. After each wash, remove the supernatant as much as possible. 3. Add 400 µL of cold Extraction Buffer A to the cell pellet. Vortex or pipette to mix, then place on ice and shake for 20-45 min. 4. Centrifuge at 4°C, 1200×g for 5 min. 5. Transfer the supernatant to another pre-cooled clean centrifuge tube. This is the cytosolic fraction. Store in refrigerator for later use or use directly for downstream experiments. 6. Resuspend the pellet in PBS, then centrifuge at 4°C, 2000×g for 5 min. Discard the supernatant. 7. The pellet is the nuclear fraction. Resuspend the pellet in 200 µL of Storage Buffer B and store for later use or use directly for downstream experiments.
Storage Buffer	Extraction Buffer A: Used for cell lysis and cytosolic fraction extraction. Storage Buffer B: Used for nuclear fraction resuspension and preservation.
Usage	For research use only. This kit is designed for the isolation of nuclear and cytosolic fractions from animal cells. The extracted fractions are suitable for downstream analyses such as transcriptional activity, RNA splicing, gel-shift assays, reporter assays, enzyme activity assays, and Western blot. The cytosolic fraction contains organelles; for cytosol without organelles, other kits should be used.

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