

Nuclei Isolation Kit

Cat. No. NUI-327K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Nuclei Isolation Kit is used for the preparation of pure nuclei and fragile nuclei from cell lines and solid tissues.
Usage	1 kit sufficient for 15 nuclei preparations (~1-10×10 ⁷ cells or 1g of tissue per preparation)
Storage	Store Nuclei Isolation Kit at 2-8 centigrade. This kit is stable for at least one year at 2-8 centigrade.
Kit Components	• Nuclei PURE Lysis Buffer, 180 ml • 10% Triton X-100, 1.7 ml • Nuclei PURE 2 M Sucrose Cushion 475 ml Solution, • Nuclei PURE Sucrose Cushion Buffer 120 ml • Nuclei PURE Storage Buffer, 90 ml
Materials Required but Not Supplied	• Cells to be used for preparation • Centrifuge (swinging bucket, refrigerated) • Beckman ultracentrifuge tubes (Ultra-Clear centrifuge tubes, 1 x 3.5 in. (25 x 89 mm), Beckman • Beckman ultracentrifuge, SW28 swinging bucket rotor and buckets (pre-cooled to 4centigrade) • Ice • Ice bucket • Small blade cell scraper

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- Dulbecco's phosphate buffered saline
- Centrifuge tubes
- Thermomixer
- Pipettes, 5 ml
- Pipettes, 10 ml
- Pipet-Aid pipette pump
- Pipette tips
- Micropipette (200 μ l)
- Microcentrifuge tubes
- 1 M Dithiothreitol (DTT) freshly prepared
- Trypan blue solution, 0.4%

Separation Protocol

Note: All manipulations should be carried out on ice or at 2-8 centigrade.

A. Harvest and Lysis of Cells Immediately before harvesting cells, make fresh Lysis Solution on ice. For each cell sample prepare:

11 ml Nuclei PURE Lysis Buffer 11 μ l 1 M dithiothreitol (DTT), freshly prepared or freshly thawed aliquot 110 μ l 10% Triton X-100

Mix, then store on ice until needed.

Procedure for Suspension Cell Lines

1. Grow cells in tissue culture flasks (e.g. 15 ml per 75 cm² flask) to desired cell density. A typical suspension cell line displaying dense growth should contain about $1-3 \times 10^7$ cells per 15 ml culture.
2. Harvest cells by transferring each culture into a separate 15 ml centrifuge tube and centrifuging at 500 X g for five minutes at 4 centigrade. Carefully aspirate the supernatant and set the cell pellet on ice.
3. Vortex the cell pellet briefly. Add 1 ml ice cold Dulbecco's phosphate buffered saline (PBS) and vortex briefly at moderate to high speed to completely suspend cells. Add an additional 9 ml of PBS, mix and set the suspension on ice. Collect the cells by centrifugation as in step 2. Carefully aspirate the clear supernatant and store the cell pellet on ice.

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4. Vortex the cell pellet briefly. Add 1 ml ice cold Lysis Solution containing DTT and Triton X-100 and vortex briefly at moderate to high speed to completely suspend cells. Add an additional 9 ml of Lysis Solution, mix well and incubate on ice for 5 minutes. Purify the nuclei by centrifugation through sucrose cushion solution as indicated below (see Section B).

Procedure for Attached Cell Lines: For most applications it is desirable to harvest the cells rapidly. For ease of manipulation and to facilitate rapid harvesting and lysis of cells, grow cells in 100 mm or 150 mm tissue culture treated Petri dishes rather than tissue culture flasks.

1. Grow cells in tissue culture treated dishes to desired cell density. A 100 mm diameter tissue culture dish of freshly confluent cells of a typical adherent cell line should contain about 0.5 to 3.0×10^7 cells per dish.
2. For each dish of cells, aspirate the medium and set the dish of cells on ice. Gently wash cells with 10 ml of ice cold Dulbecco's phosphate buffered saline (PBS). Carefully aspirate the wash solution.
3. Add 10 ml of ice cold Lysis Solution containing DTT and Triton X-100 to each dish. Harvest and lyse cells by thoroughly scraping each dish with a small bladed cell scraper. Transfer the entire cell lysate from each plate to a separate 15 ml centrifuge tube, vortex briefly, and incubate on ice for five minutes or until cells have been harvested from all culture dishes. Purify the nuclei by centrifugation through sucrose cushion solution as indicated below (see Section B.).

Procedure for Solid Tissues:

1. Dissect fresh tissue from a sacrificed animal. Use about 1 g of tissue per preparation. Rinse the tissue briefly with ice cold Dulbecco's phosphate buffered saline (PBS) and blot dry. Keep the tissue sample on ice in a suitable container if mincing (step 2) is not performed immediately.
2. Transfer each tissue sample to a small plastic Petri dish on ice and mince thoroughly with a sharp scalpel.
3. Transfer each minced tissue sample to a suitable chilled container with 10 ml ice cold Lysis Solution containing DTT and Triton X-100 and homogenize at 2-

8centigrade to lyse the cells completely and release intact nuclei. Cell lysis and nuclei morphology can be determined by microscopic examination to insure proper homogenization. For data shown below, minced tissue was transferred to 50 ml plastic centrifuge tubes on ice and tissue was homogenized for 30 to 45 seconds with an Omni International 1000 homogenizer on high setting (20,000 rpm). Purify nuclei by centrifugation 3 through sucrose cushion solution as indicated below (see Section B).

B. Purification of Nuclei by Centrifugation through Sucrose Cushion

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. A concentration of 1.8 M sucrose in the sucrose cushion solution was used for all the cell lines and tissues tested for the data presented below, and is recommended for routine use in this procedure. The sucrose concentration can be adjusted as needed, as indicated in the sucrose concentration table below (Table 1).

Immediately before use, make fresh 1.8 M Sucrose Cushion Solution on ice. For each cell sample prepare:

27 ml Nuclei PURE 2 M Sucrose Cushion Solution 3 ml Nuclei PURE Sucrose Cushion Buffer 30 μ l 1 M dithiothreitol (DTT), freshly prepared or freshly thawed aliquot Mix and store on ice until needed.

Procedure for All Lysates

1. To each 10 ml lysate sample on ice add 18 ml of cold 1.8 M Sucrose Cushion Solution. Gently mix well and set on ice.
2. For each sample preparation, add 10 ml of ice cold 1.8 M Sucrose Cushion Solution to the bottom of a labeled Beckman ultracentrifuge tube (Ultra-Clear Centrifuge Tubes, 1 x 3.5 in. (25 x 89 mm), Beckman) on ice.
3. Carefully and slowly layer the 28 ml of lysate solution from step 1 on top of the 10 ml of Sucrose Cushion Solution from step 2. Avoid disturbing the Sucrose Cushion

layer. This can be done at the ultracentrifuge immediately before loading the ultracentrifuge rotor. Check the balance of the filled ultracentrifuge tubes and adjust as necessary.

4. Carefully place the filled ultracentrifuge tubes into pre-cooled buckets of a Beckman Ultracentrifuge SW28 swinging bucket rotor. Place the buckets in a pre-cooled SW28 rotor and centrifuge samples for 45 minutes at 30,000 X g (13,000 rpm) at 4 centigrade.

5. Remove the sample tubes from the ultracentrifuge and set on ice. The nuclei should be visible as a small, thin pellet at the bottom of each tube. 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. Vortex the nuclei pellet briefly, add 1 ml cold Nuclei PURE Storage Buffer and vortex again to completely resuspend the nuclei pellet. Add an additional 4 ml of Nuclei PURE Storage Buffer and vortex briefly. Set on ice.

7. Collect the nuclei by centrifugation at 500 X g for five minutes at 4centigrade. Carefully aspirate the clear supernatant from each tube and set the nuclei pellet on ice.

8. Vortex the nuclei pellet briefly, add 200 µcold Nuclei PURE Storage Buffer and vortex again to completely suspend the nuclei pellet. Set on ice. Triturate (pipet up and down) 5-10 times with a micropipette to help break up clumps of nuclei. Carefully transfer the final suspension of nuclei in Nuclei PURE Storage Buffer to a microcentrifuge tube for storage. If desired, take a small sample to dilute for counting (see below). Nuclei should be used immediately or frozen at -70centigrade for storage. Nuclei frozen at -70 centigrade in Nuclei PURE Storage Buffer are stable for at least 3 months.