Nuclei Isolation Kit

Cat. No. NUI-326K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Nuclei Isolation Kit is designed for the rapid isolation of nuclei from mammalian cells.
Applications	Suitable as a source of nuclear components, to produce nuclei for in vitro apoptosis assays, and for functional studies.
Usage	1 kit contains reagents sufficient for 25 preparations.
Storage	Store the Kit at 2-8 centigrade. This kit is stable for at least one year at 2-8 centigrade.
Kit Components	Nuclei EZ Lysis Buffer, 200 ml Nuclei EZ Storage Buffer, 5 ml
Materials Required but Not Supplied	• Cells to be used for preparation • Centrifuge (swinging bucket, refrigerated) • Ice • Ice Bucket • Small Blade Cell Scraper • Dulbecco's Phosphate Buffered Saline • Centrifuge Tubes • Vortex Mixer • Pipettes, 5 ml • Pipettes, 10 ml

Tel: 1-631-559-9269 1-516-512-3133

Email: info@creative-biomart.com Fax: 1-631-938-8127

45-1 Ramsey Road, Shirley, NY 11967, USA

For Research Use Only

	• Pipet-Aid pipette pump • Pipette tips • Micropipette (200 µl) • Microcentrifuge Tubes • Trypan Blue Solution (0.4%)
Separation Protocol	Note: To help ensure good quality nuclei preparation, perform the isolation procedure quickly and keep samples cold. All manipulations should be carried out on ice or at 2-8 centigrade. Procedure for Attached Cell Lines: For most applications it is desirable to harvest 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. A typical isolation of nuclei with this kit can be easily done in less than one hour. 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 x 10^7 cells per dish. 2. Wash cells as follows. 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. Harvest and lyse cells as follows. Add 4 ml of ice cold Nuclei EZ lysis buffer 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 set on ice for five minutes or until cells have been harvested from all culture dishes. 4. Collect the nuclei by centrifugation at 500 x g for five minutes at 4 centigrade. Carefully aspirate the clear supernatant from each tube and set the nuclei pellet on ice. Note: The supernatant contains cytoplasmic components and can be saved for later analysis or use.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

5. Resuspend and wash nuclei in 4 ml of ice cold Nuclei EZ lysis buffer as follows. Vortex nuclei pellet briefly. Add 0.5 ml cold Nuclei EZ lysis buffer and vortex briefly at moderate to high speed to completely suspend nuclei pellet. Add the remaining 3.5 ml of Nuclei EZ lysis buffer, mix well and set on ice for 5 minutes.

6. Collect washed nuclei by centrifugation as in step 4. Carefully aspirate the clear supernatant and set the nuclei pellet on ice.

7. Resuspend each nuclei pellet in 200 µof ice cold Nuclei EZ storage buffer as follows. Vortex pellet briefly, add 200 µcold Nuclei EZ storage buffer and vortex as above to completely suspend the nuclei pellet. Set on ice. Triturate (pipette up and down) 5-10 times with a micropipette to help break up clumps of nuclei. Carefully transfer the final nuclei suspension in storage buffer to a microcentrifuge tube for storage.

Take a small sample to dilute for counting (see below). Nuclei should be used immediately or frozen at -70 centigrade for storage. Nuclei frozen at -70 centigrade in Nuclei EZ storage buffer are stable for at least several months.

Procedure for Suspension Cell Lines:

1. Grow cells in tissue culture flasks (15 ml per 75 cm2 flask) to desired cell density.

2. Harvest cells as follows. Transfer each culture into a separate 15 ml centrifuge tube and centrifuge at 500 x g for five minutes at 4 centigrade. Carefully aspirate the supernatant and set the cell pellet on ice.

3. Wash cells in 10 ml of ice cold Dulbecco’s Phosphate Buffered Saline (PBS) as follows. Vortex cell pellet briefly. Add 1 ml cold PBS and vortex briefly at moderate to high speed to completely suspend cells. Add remaining 9 ml of PBS, mix and set on ice. Collect cells by centrifugation as in step 2. Carefully aspirate clear supernatants and set cell pellets on ice.

4. Lyse cells in 4 ml of ice cold Nuclei EZ lysis buffer as follows. Vortex pellet briefly. Add 0.5 ml cold Nuclei EZ lysis buffer and vortex briefly at moderate to high speed to completely suspend cells. Add the remaining 3.5 ml of Nuclei EZ lysis buffer, mix well and set on ice for 5 minutes.

5. Collect the nuclei by centrifugation at 500 x g for five minutes at 4 centigrade.

	Carefully aspirate the clear supernatant from each tube and set the nuclei pellet on ice. Note: The supernatant contains cytoplasmic components and can be saved for later analysis or use. 6. Resuspend and wash nuclei in 4 ml of ice cold Nuclei EZ lysis buffer as follows. Vortex nuclei pellet briefly. Add 0.5 ml cold Nuclei EZ lysis buffer and vortex briefly at moderate to high speed to completely suspend nuclei pellet. Add the remaining 3.5 ml of Nuclei EZ lysis buffer, mix well and set on ice for 5 minutes. 7. Collect washed nuclei by centrifugation as in step 5. Carefully aspirate the clear supernatant and set the nuclei pellet on ice. 8. Resuspend each nuclei pellet in 200 μof ice cold Nuclei EZ storage buffer as follows. Vortex pellet briefly, add 200 μcold Nuclei EZ storage buffer and vortex as above to completely suspend nuclei pellet. Set on ice. Triturate (pipette up and down) 5-10 times with a micropipette to help break up clumps of nuclei. Carefully transfer the final nuclei suspension in storage buffer to a microcentrifuge tube for storage. Take a small sample to dilute for counting (see below). Nuclei should be used immediately or frozen at -70 centigrade for storage. Nuclei frozen at -70 centigrade in Nuclei EZ storage buffer are stable for at least several months.

Tel: 1-631-559-9269　1-516-512-3133
Email: info@creative-biomart.com　Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

For Research Use Only