3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

NUCLEI EZ PREP NUCLEI ISOLATION KIT

Product Number **NUC-101**
Store at 2-8 °C

TECHNICAL BULLETIN

Product Description
Sigma's Nuclei EZ Prep Kit is designed for the rapid isolation of nuclei from mammalian cells. The simple protocol provides a high yield of nuclei from commonly used cell types, including adherent (HEK293 and COS7) and non-adherent (Jurkat and HFN7.1) tissue culture cell lines, and peripheral blood mononuclear cells (PBMCs). Kit components are nuclease and protease-free. The isolated nuclei can be preserved frozen several months in the included storage buffer. These preparations are suitable for many cell biology applications, e.g. as a source of nuclear components, such as chromatin, genomic DNA, histones and nuclear RNA/RNP, to produce nuclei for *in vitro* apoptosis assays, and for functional studies such as examination of the transcriptional status of cells. One kit contains reagents sufficient for 25 preparations.

Components

- Nuclei EZ Lysis Buffer, Product No. N 3408 — 200 ml
- Nuclei EZ Storage Buffer, Product No. S 8933 — 5 ml

Reagents and Equipment Required But Not Provided
(Sigma Product Numbers have been given where appropriate)

- Cells to be used for preparation
- Centrifuge (swinging bucket, refrigerated)
- Ice
- Ice Bucket, Product No. Z37,932-8
- Small Blade Cell Scraper, Product No. C 2802
- Dulbecco's Phosphate Buffered Saline, Product No. D 8537
- Centrifuge Tubes, Product No. C 8046
- Vortex Mixer, Product No. Z36,815-6
- Pipettes, 5 ml, Product No. P 3672
- Pipettes, 10 ml, Product No. P 3797
- Pipet-Aid pipette pump, Product No. P 6175
- Pipette tips, Product No. P 0310
- Micropipette (200 µl), Product No. Z36,811-3
- Microcentrifuge Tubes, Product No. T 9661
- Trypan Blue Solution (0.4%),Product No. T 8154

Precautions and Disclaimer
Sigma's Nuclei EZ Prep Kit is for laboratory use only, not for drug, household or other uses.

Storage/Stability
Store the Nuclei EZ Prep Kit at 2-8 °C. This kit is stable for at least one year at 2-8 °C.

Procedure
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 °C.

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 (Product No. C 6546 or C 6671), 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 °C. 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.

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 µl of ice cold Nuclei EZ storage buffer as follows. Vortex pellet briefly, add 200 µl cold 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 °C for storage. Nuclei frozen at –70 °C in Nuclei EZ storage buffer are stable for at least several months.

<u>Procedure for Suspension Cell Lines:</u>

1. Grow cells in tissue culture flasks (15 ml per 75 cm^2 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 °C. 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 °C. 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 µl of ice cold Nuclei EZ storage buffer as follows. Vortex pellet briefly, add 200 µl cold 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 °C for storage. Nuclei frozen at –70 °C in Nuclei EZ storage buffer are stable for at least several months.

Results

Quality of nuclei

Nuclei isolated using the Nuclei EZ Prep Kit were capable of synthesizing mRNA, as determined by [α-^{32}P]GTP incorporation in the absence and presence α-amanitin at a concentration known to selectively inhibit RNA polymerase II (Reference 1). See Table 1.

Table 1
mRNA synthesis by Jurkat nuclei isolated using the Nuclei EZ Prep Kit

α-amanitin (0.25 µg/ml)	[α-^{32}P]GTP incorporation[1] cpm/10^7 nuclei (x 10^{-5})
-	5.56 (± 0.13)
+	1.54 (± 0.04)

[1] TCA precipitable counts were determined after labeling 1.3 x 10^6 nuclei in a 40 µl reaction one hour at 25 °C with 50 µCi/ml [α-^{32}P]GTP.

Yield of nuclei

The final number of nuclei can be determined by dilution in trypan blue solution and counting with a hemacytometer (Product No. Z35,962-9). Since the Nuclei EZ storage buffer contains glycerol, nuclei in this buffer may swell if diluted using aqueous solutions. Therefore, it is recommended that the final nuclei in storage buffer be diluted for counting into a trypan blue solution which has been previously diluted (1 to 4) in storage buffer or in a 30% glycerol solution to prevent swelling of nuclei.

The nuclei yield can be determined by comparing the number of final nuclei to starting cell number determined by hemacytometer counting. For suspension cells, samples of the cultures can be directly diluted into trypan blue counting solution and counted before beginning the nuclei isolation procedure. For adherent cells, separate representative plates should be trypsinized (to release all cells), diluted in trypan blue counting solution, and counted. Typical counts and nuclei yields from several commonly used cell lines and peripheral blood mononuclear cells (PBMCs) are shown in Table 2.

The purity of the final nuclei can be quickly determined by careful visual microscopic inspection of the nuclei diluted in trypan blue counting solution as described above. 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.

Table 2
Typical yield of nuclei isolated from commonly used cells using the Nuclei EZ Prep Kit [1]

Cells	Cell Type	Average # Cells[3] x 10^{-7}	Average # Nuclei x 10^{-7}	Average % Yield
CHO	Hamster ovary, tissue culture cell line (adherent)	1.0 (± 0.2)	1.0 (± 0.4)	98 (± 24)[4]
HEK293	Embryonic kidney, tissue culture cell line (adherent)	2.3 (± 0.5)	2.1 (± 0.6)	90 (± 20)[4]
HFN7.1	Hybridoma, tissue culture cell line (suspension)	3.8 (± 1.0)	2.5 (± 0.7)	65 (± 2)
Jurkat	T cell leukemia, tissue culture cell line (suspension)	2.6 (± 0.1)	1.6 (± 0.1)	62 (± 7)
PBMC[2]	Peripheral blood mononuclear cells	5.0 (± 2.1)	3.0 (± 1.0)	63 (± 6)

[1] Data show average results of three separate nuclei isolation experiments for each cell type. Standard errors are indicated in parentheses.
[2] PBMC, peripheral blood mononuclear cells were isolated from blood by Histopaque®-1077 gradient centrifugation using the Accuspin™ System (Product No. A 7054) prior to nuclei isolation.
[3] The data for adherent cells represents preparations each from one 100 mm tissue culture dish of freshly confluent cells. Representative plates were trypsinized and counted for total cell numbers. The data from suspension cells represents preparations each from 15 ml suspension cultures grown in 75 cm^2 tissue culture flasks. Each PBMC preparation was from about 35 ml freshly donated human blood.
[4] Standard error was greater with adherent cell lines due to variability in counting cells and recovery of lysates from culture dishes.

Troubleshooting Guide

Problem:	Cause:	Solution:
Low Yield Yield will vary between cell lines, but should typically be greater than 30% (see Table 2 for examples).	Poor recovery from attached cells at harvest/lysis step	Scrape plates well and examine plates after removing lysates to ensure that a vast majority of the cells were removed.
	Incomplete lysis	Some adherent cell lines, such as MDCK cells, that adhere very tightly to each other are difficult to lyse. Try homogenizing cells after scraping from plates in lysis buffer.
	Accidental aspiration of nuclei pellets after centrifugation	Aspirate lysate and wash supernatants very carefully after centrifugation. It may be helpful to aspirate through a narrow, thin tube such as a micropipette tip or syringe needle.
	Poor recovery of final nuclei pellets	Suspend the final nuclei pellet completely and make sure that no residual nuclei are left in the centrifuge tube before discarding.
	Clumping of nuclei	Excessive nuclei clumping can make the final nuclei difficult to count. DNA released from damaged nuclei can cause excessive clumping. To minimize damage to nuclei, isolate nuclei rapidly and keep cold during isolation procedure. Gently triturating final nuclei several times can help to suspend and disperse nuclei.
Impure Nuclei (Contamination with non-nuclear material)	Poor lysis	Some adherent cell lines, such as MDCK cells, that adhere very tightly to each other are difficult to lyse. Try briefly homogenizing cell suspension after scraping from plates in lysis buffer.
	Incomplete aspiration of supernatants after centrifugation	Aspirate lysate and wash supernatants very carefully after centrifugation. It may be helpful to aspirate through a narrow, thin tube such as a micropipette tip or syringe needle.
	Clumping of nuclei.	Excessive nuclei clumping can trap cytoplasmic debris. DNA released from damaged nuclei can cause excessive clumping. To minimize damage to nuclei, isolate nuclei rapidly and keep cold during isolation procedure. Gently triturating nuclei several times when suspending after centrifugation can help to suspend and disperse nuclei.
	Poor purity of nuclei after washing	If nuclei appear to have cellular debris contamination after the wash step, perform an additional wash incubation and centrifugation in lysis buffer. Nuclei from most tissues and some strongly adherent cell lines are more difficult to purify. Isolation of such nuclei often requires an additional purification step of ultracentrifugation through a dense sucrose cushion. This step helps remove tightly bound cytoplasmic components from the nuclei. To help ensure good quality nuclei preparation, perform the isolation procedure quickly and keep samples cold.
	Possible degradation of desired cellular components	Protease, nuclease, phosphatase or other inhibitors can be added as appropriate to the Nuclei EZ Lysis Buffer.

References

1. "Analysis of nuclear RNA", Chapter 19, in RNA Methodologies: A Laboratory Guide for Isolation and Characterization, Robert E. Farrell, Jr., Academic Press, San Diego, p. 406-437 (1998)
2. Greenberg, M.E. and Bender, T.P., "Identification of newly transcribed RNA", in Current Protocols in Molecular Biology, Ausubel, F.M., *et al*., (Eds.), John Wiley and Sons, New York, p. 4.10.1-4.10.11 (1987)
3. Marzluff, W.F. and Huang, R.C.C., "Transcription of RNA in isolated nuclei", Chapter 4, in Transcription and Translation: A Practical Approach, Hames, B.D. and Higgens, S.J., (Eds.), IRL Press, Oxford, UK, p. 89-129 (1984)

Lpg 01/03