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ProductInformation

NUCLEI PURE PREP NUCLEI ISOLATION KIT

Product No. **NUC-201**
Technical Bulletin No. MB-735
Store at 2-8 °C

TECHNICAL BULLETIN

Product Description
Sigma's Nuclei PURE Prep Nuclei Isolation Kit is designed for the preparation of pure nuclei and fragile nuclei from cell lines and solid tissues. The simple protocol incorporates centrifugation through a dense sucrose cushion to protect nuclei and strip away cytoplasmic contaminants. This kit has been used to purify nuclei from common cell lines (Jurkat, HFN7.1, COS7, HEK293 and MDCK) and tissues (spleen and liver). Nuclei purified from Jurkat cells and rat liver tissue with the Nuclei PURE Prep kit were capable of synthesizing RNA. The isolated nuclei can be preserved frozen in the included storage buffer. These preparations are a suitable source for nuclear components, such as chromatin, genomic DNA, histones, and nuclear RNA/RNP. The kit may also be used to produce nuclei for *in vitro* apoptosis assays**,** and for functional studies, such as examination of the transcriptional status of cells. Kit components are nuclease and protease free.

Components
Sufficient for 15 nuclei preparations (~ 1-10 x 10^7 tissue culture cells or 1 g of tissue per preparation)

- Nuclei PURE Lysis Buffer, Product No. L9286 — 180 ml
- 10% Triton X-100, Product No. T1565 — 1.7 ml
- Nuclei PURE 2 M Sucrose Cushion Solution, Product No. S9308 — 475 ml
- Nuclei PURE Sucrose Cushion Buffer Product No. S9058 — 120 ml
- Nuclei PURE Storage Buffer, Product No. S9183 — 90 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)
- Beckman ultracentrifuge tubes (Ultra-Clear centrifuge tubes, 1 x 3.5 in. (25 x 89 mm), Beckman Catalog #344058; 40 ml capacity)
- Beckman ultracentrifuge, SW28 swinging bucket rotor and buckets (pre-cooled to 4°C)
- Ice
- Ice bucket, Product No. Z37,932-8
- Small blade cell scraper, Product No. C2802
- Dulbecco's phosphate buffered saline, Product No. D8537
- Centrifuge tubes, Product No. C8046
- Thermomixer, Product No. Z36,815-6
- Pipettes, 5 ml, Product No. P3672
- Pipettes, 10 ml, Product No. P3797
- Pipet-Aid pipette pump, Product No. P6175
- Pipette tips, Product No. P0310
- Micropipette (200 µl), Product No. Z36,811-3
- Microcentrifuge tubes, Product No. T9661
- 1 M Dithiothreitol (DTT) freshly prepared from Product No. D9779 or fresh aliquot from solution stored at -20°C
- Trypan blue solution, 0.4%, Product No. T8154

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

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

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

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 µl 1 M dithiothreitol (DTT), freshly prepared or freshly thawed aliquot
110 µl 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 cm^2 flask) to desired cell density. A typical suspension cell line displaying dense growth should contain about 1-3 x 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 °C. 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.

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 (Product No. C 6546 or C 6671) 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 x 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 2-8°C 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

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 µl 1 M dithiothreitol (DTT), freshly prepared or freshly thawed aliquot

Mix and store on ice until needed.

Table 1
Formulations for various sucrose cushion concentrations (30 ml per sample)

Sucrose Cushion Concentration	2 M Sucrose Cushion Solution Volume (ml)	Sucrose Cushion Buffer Volume (ml)
2.00 M	30	0
1.95 M	29.25	0.75
1.90 M	28.5	1.5
1.85 M	27.75	2.25
1.80 M	**27**	**3**
1.75 M	26.25	3.75
1.70 M	25.5	4.5

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 Catalog #344058; 40 ml capacity) 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 °C.
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 4°C. Carefully aspirate the clear supernatant from each tube and set the nuclei pellet on ice.
8. Vortex the nuclei pellet briefly, add 200 µl cold 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 -70°C for storage. Nuclei frozen at –70 °C in Nuclei PURE Storage Buffer are stable for at least 3 months.

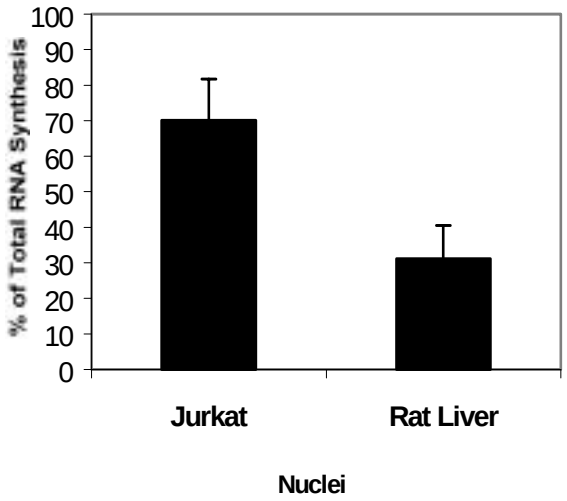
Results

Quality of nuclei

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

Figure 1
***in vitro* mRNA synthesis by Jurkat and rat liver nuclei isolated using the Nuclei PURE Prep Kit**

[1] TCA precipitable counts were determined after labeling



nuclei in a 40 µl reaction volume for 45 minutes at 25°C with 50 µCi/ml [α-^{32}P]GTP. For Jurkat cells, 1.4-2.8 x 10^6 nuclei were used and 100% RNA synthesis values ranged from 3.1-45.1 pM GTP/10^6 nuclei. For rat liver cells, 1.4-7.3 x 10^6 nuclei were used and 100% RNA synthesis values ranged from 4.5-7.3 pM GTP/10^6 nuclei.

Yield of nuclei

The final number of nuclei can be determined by dilution in trypan blue solution (Product No. T 8154) and counting with a hemacytometer (Product No. Z35,962-9). Since the Nuclei PURE Storage Buffer contains glycerol, nuclei in this buffer may swell if diluted using aqueous solutions. Therefore, it is recommended that nuclei in Nuclei PURE Storage Buffer be diluted for counting into a trypan blue solution which has been previously diluted (1 to 4) in Nuclei PURE 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 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 solution, and counted. For tissues, nuclei yields can be estimated per gram of tissue. Typical counts and nuclei yields from several commonly used cell lines and tissues are shown in Tables 2 and 3.

The purity of the final nuclei can be quickly determined by careful visual microscopic inspection of the nuclei diluted in trypan blue solution as described above. Nuclei will stain blue with a uniform circular or sausage-shaped appearance, whereas cytoplasmic contamination and cell debris, if present, will stain light blue with an irregular morphology and will be clearly visible.

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

Cells	Cell Type	Average # Cells[2] x 10^{-7}	Average # Nuclei x 10^{-7}	Average % Yield
Jurkat	Human T cell leukemia, tissue culture cell line (suspension)	4.3 (± 2.2)	2.0 (± 1.6)	48 (± 36)
HFN7.1	Hybridoma, tissue culture cell line (suspension)	4.5 (± 3.4)	2.4 (± 2.1)	49 (± 16)
COS7	African green monkey kidney, tissue culture cell line (adherent)	1.8 (± 0.4)	0.8 (± 0.4)	50 (± 29)
HEK293	Human embryonic kidney, tissue culture cell line (adherent)	3.2 (± 0.4)	1.5 (± 0.4)	48 (± 12)
MDCK	Madin-Darby canine kidney, tissue culture cell line (adherent)	2.9 (± 1.1)	0.5 (± 0.2)	19 (± 10)

[1] Data show average results of at least three separate nuclei isolation experiments for each cell type. Standard errors are indicated in parentheses.
[2] 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.

Table 3
Yield of nuclei isolated from mammalian tissues using the Nuclei PURE Prep Kit [1]

Tissue	Homogenate[2] Average # Nuclei/gram X 10^{-7}	Purified Nuclei Average # Nuclei/gram x 10^{-7}	Average % Yield
Rat Liver	15.2 (± 5.2)	6.2 (± 1.7)	43.1 (± 14.1)
Rat Spleen	31.8 (± 5.3)	18.4 (± 6.4)	59.5 (± 23.2)

[1] Data show average results of at least three separate nuclei isolation experiments for each tissue type. Standard errors are indicated in parentheses.
[2] Homogenates were made Omni International 1000 homogenizer according to the procedure in Section A above, using 0.8-1.6 g of freshly dissected tissue for each preparation. It is difficult to count accurately the number of nuclei released after homogenization, because of extensive amounts of tissue and cellular debris in tissue homogenate samples.

Troubleshooting Guide

Problem	Cause	Solution
Low Yield Yield will vary between cell lines, but should typically be greater than 30% (see Table 2 and 3 for examples)	Poor recovery from attached cells at harvest/lysis step	Scrape plates well and examine plates after removing lysates to ensure that essentially all 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. After scraping the cells from the plates, try homogenizing the cells in lysis buffer.
	Accidental aspiration of nuclei pellets after centrifugation	Aspirate supernatants very carefully after centrifugation steps. It may be helpful to aspirate through a narrow, thin tube such as a micropipette tip or syringe needle.
	Poor recovery of nuclei pellets	Suspend nuclei pellets completely and make sure that no residual nuclei are left in centrifuge tubes 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. After scraping the cells from the plates, try briefly homogenizing the cell suspension 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	Nuclei from most tissues and some strongly adherent cell lines are more difficult to purify. The procedure described here is designed for isolation of such nuclei by utilizing a purification step of ultracentrifugation through a dense sucrose cushion. This step helps remove tightly bound cytoplasmic components from the nuclei. Handle sucrose cushion tubes carefully to avoid disturbing the sucrose cushion. 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 PURE Lysis Buffer.

References

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