

## Abstract

Preparation of nuclei is often the first step in studying nuclear components and events. An improved method of preparing nuclei from adherent mammalian tissue culture cells by simultaneous harvest and lysis of cells was developed and characterized. The modified procedure resulted in rapid and reliable recovery of nuclei. Nuclei were isolated from adherent and non-adherent tissue culture cells and monocytes by using a hypotonic lysis buffer. Nuclei purity was tested by cytological staining, SDS-PAGE and electron microscopic analysis. The isolated nuclei were functional for synthesis of RNA polymerase II dependent transcripts. In addition, active transcription of nuclear genes was tested by nuclear transcription assays.

Nuclei produced by this method should be useful for many applications in biochemistry and cell biology, as a source of nuclear components, for *in vitro* apoptosis assays and for functional studies on the transcriptional status of cells.

## Introduction

**Goal** – To develop simple, reliable methods for preparing functional nuclei from mammalian cells for applications in biochemistry and cell biology.

**Approach** – To test and improve existing techniques for:

- 1) Nuclei isolation from tissue culture cells
- 2) Nuclei isolation from mammalian tissues

## Isolation of Nuclei from Cultured Cells

Nuclei isolated from mammalian cells are useful for many 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, for functional studies, such as examination of the transcriptional status of cells, and for proteomic studies of the cell nucleus.

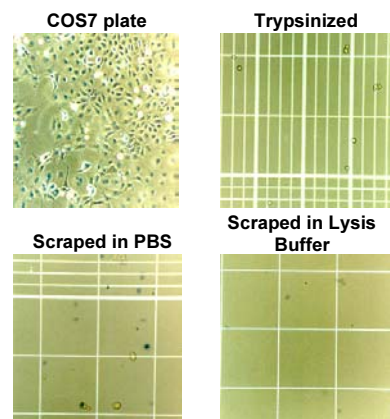
We developed an improved version (Nuclei EZ Prep™ nuclei isolation kit) of standard methods (5,6,7) for small-scale preparation of functional nuclei from mammalian cells. The improved method allows rapid, **simultaneous harvest and lysis** of cells in a hypotonic buffer containing a gentle, nonionic detergent, Igepal CA-630 (NP-40).

## Nuclei Isolation Method

Adherent Cells	Suspension Cells
Aspirate medium	Collect cells (centrifuge)
PBS wash/aspirate	PBS wash/spin/aspirate
Scrape in lysis buffer*	Suspend in lysis buffer
Incubate 5 min, 4° C	Incubate 5 min, 4° C
Collect nuclei (5 min, 500 x g)	Collect nuclei (5 min, 500 x g)
Lysis buffer wash	Lysis buffer wash
Collect nuclei (5 min, 500 x g)	Collect nuclei (5 min, 500 x g)
Store in storage buffer (-70° C, >6 months)	Store in storage buffer (-70° C, >6 months)

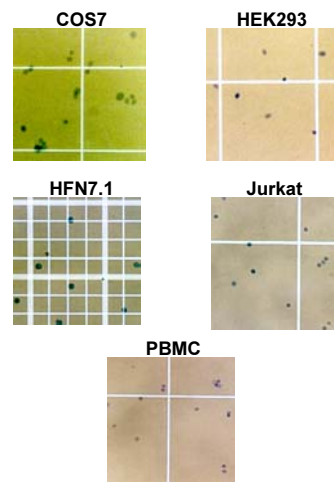
\* Replaces harvesting cells by scraping in PBS.

## Harvest and Lysis of Adherent Cells



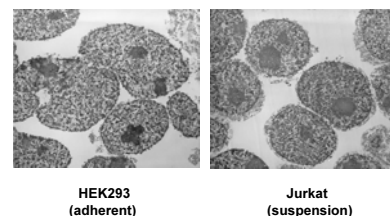
**Fig. 1.** Scraping adherent cells directly in lysis buffer results in rapid and simultaneous harvest and lysis of cells.

## Purified Nuclei



**Fig. 2.** Morphology of purified nuclei after storage at -70° C.

## Electron Micrographs of Purified Nuclei



**Fig. 3.** Nuclei were fixed, sectioned, stained and visualized by transmission EM.

## Nuclei Yields

Table 1. Yield of nuclei isolated from commonly used cells using the Nuclei EZ Prep™ Kit (Sigma Product Number NUC-101).<sup>1</sup>

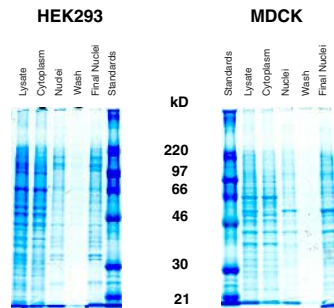
Cells <sup>2</sup>	Cell Type	Average # Cells <sup>3</sup> x 10 <sup>7</sup>	Average # Nuclei x 10 <sup>7</sup>	Average % Yield
CHO	Hamster ovary, tissue culture cell line (adherent)	1.0 (±0.2)	1.0 (±0.4)	98 (±24)
HEK293	Embryonic kidney, tissue culture cell line (adherent)	2.3 (±0.5)	2.1 (±0.6)	90 (±20)
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	Peripheral blood mononuclear cells	5.0 (±2.1)	3.0 (±1.0)	63 (±6)

<sup>1</sup> Data show average results of three separate nuclei isolation experiments for each cell type. Standard errors are indicated in parentheses.

<sup>2</sup> PBMC, peripheral blood mononuclear cells isolated from blood by Histopaque®-1077 gradient centrifugation using the Accuspin™ System (Sigma Product Number A 7054).

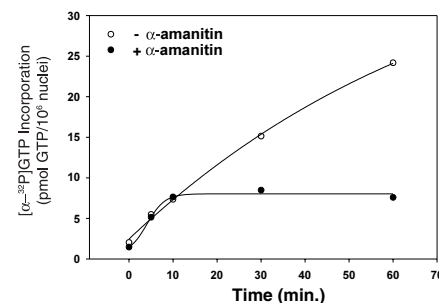
<sup>3</sup> The data for adherent cells represents preparations each from one 100 mm tissue culture dish of freshly confluent cells. Representative plates were trypsinized and counted for total cell numbers. The data from suspension cells represents preparations each from 15 ml suspension cultures grown in T75 tissue culture flasks. Each PBMC preparation was from about 35 ml freshly donated human blood.

## Protein Distribution



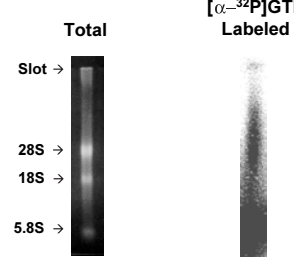
**Fig. 4.** Fractions taken during nuclei isolation were analyzed by SDS-PAGE and visualized by Coomassie Blue staining.

## mRNA Synthesis



**Fig. 5.** Nuclei were isolated from Jurkat cells and incubated without (open circles) and with (closed circle) 0.25 µg/ml α-amanitin at 25° C in [α-<sup>32</sup>P]GTP labeling mix (ref. 5). Samples were taken at times indicated, and TCA precipitable counts were determined.

## RNA Isolated from Nuclei



**Fig. 6.** Nuclei were incubated 1 hr. at 25° C with [α-<sup>32</sup>P]GTP. The nuclei were lysed in 1% SDS, 10 mM Na<sub>2</sub>EDTA, pH 7.0 and extracted with phenol/chloroform. The RNA was ethanol precipitated, dissolved, purified on a G50 spin-column and run on a 1.2% agarose RNA gel. The RNA was visualized by EtBr staining (left) and phosphorimaging (right). The <sup>32</sup>P-labeled RNA run-off transcripts can be seen on the right.

## Discussion

- 1) The Nuclei EZ Prep™ nuclei isolation kit was suitable for isolation of nuclei with high yield and purity from cultured mammalian cells.
- 2) The improved method has the advantage of rapid and simultaneous harvest and lysis of cells.
- 3) Nuclei isolated by this method are functional for synthesis of run-off mRNA transcripts.

## Isolation of Nuclei from Tissues

Functional, pure nuclei are more difficult to isolate from animal tissues, from cultured cells with fragile nuclei or from cultured cells that are difficult to harvest or lyse (e.g., epithelial-like cells with tight junctions).

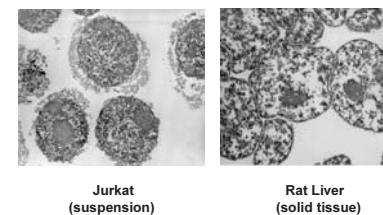
We developed a modified version (Nuclei PURE Prep™ nuclei isolation kit) of standard methods (5,6,7) to allow isolation of functional nuclei from a variety of cultured cells and animal tissues. The modified method employs purification of nuclei by **ultracentrifugation through a 1.8 M sucrose cushion**.

## Nuclei Isolation Using Sucrose Cushion

- Collect and wash cells/tissue
- Homogenize in lysis buffer (iso-osmotic sucrose, 0.1% Triton X-100)
- Purify through 1.8 M sucrose cushion\* (45 min., 30,000 x g)
- Storage buffer wash
- Collect nuclei (5 min., 500 x g)
- Store in storage buffer (-70° C, >6 months)

\* Replaces purification through a 2.0 M sucrose cushion.

## Electron Micrographs of Nuclei Purified with 1.8 M Sucrose Cushions



**Fig. 7.** Nuclei were fixed, sectioned, stained and visualized by transmission EM.

## Nuclei Yields

Table 2. Yields of nuclei isolated from commonly used cells using the Nuclei PURE Prep™ Kit (Sigma Product Number NUC-201).<sup>1</sup>

Cells	Cell Type	Average # Cells <sup>2</sup> x 10 <sup>7</sup>	Average # Nuclei x 10 <sup>7</sup>	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)

<sup>1</sup> Data show average results of three separate nuclei isolation experiments for each cell type. Standard errors are indicated in parentheses.

<sup>2</sup> 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<sup>2</sup> tissue culture flasks.

## Nuclei Yields

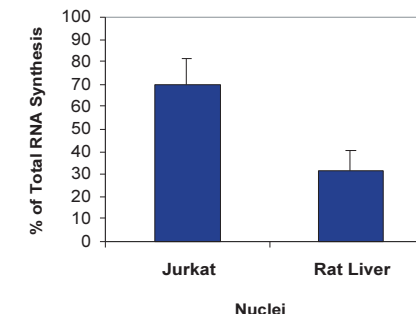
Table 3. Yields of nuclei isolated from mammalian tissues using the Nuclei PURE Prep™ Kit<sup>1</sup>

Tissue	Homogenate <sup>2</sup> Average # Nuclei/gram x 10 <sup>7</sup>	Purified Nuclei Average # Nuclei/gram x 10 <sup>7</sup>	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)

<sup>1</sup> Data show average results of at least three separate nuclei isolation experiments for each cell type. Standard errors are indicated in parentheses.

<sup>2</sup> Homogenates were made with an Omni International 1000 homogenizer, 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.

## mRNA Synthesis



**Fig. 8.** *in vitro* mRNA synthesis by Jurkat and rat liver nuclei isolated using the Nuclei PURE Prep™ Kit. TCA precipitable counts were determined after labeling nuclei in a 40 µl reaction volume (± 0.25 µg/ml α-amanitin) for 45 minutes at 25° C with 50 µCi/ml [α-<sup>32</sup>P]GTP. For Jurkat cells, 1.4-2.8 x 10<sup>6</sup> nuclei were used and 100% RNA synthesis values ranged from 3.1-45.1 pM GTP/10<sup>6</sup> nuclei. For rat liver cells, 1.4-7.3 x 10<sup>6</sup> nuclei were used and 100% RNA synthesis values ranged from 4.5-7.3 pM GTP/10<sup>6</sup> nuclei.

## Discussion

- 1) The Nuclei PURE Prep™ nuclei isolation kit was suitable for isolation of nuclei with high yield and purity from cultured mammalian cells and tissues.
- 2) The modified method has the advantage of a sucrose cushion formulation that is applicable to many different cell and tissue types.
- 3) Nuclei isolated by this method are functional for synthesis of run-off mRNA transcripts.

## Conclusion

- 1) Existing techniques were modified and improved to yield simple, reliable methods for preparing functional, pure nuclei from a variety of mammalian cell lines and tissues.
- 2) Pure and functional nuclei produced by these methods will be increasing useful for a number of applications in cell biology and biochemistry, including studies on the transcriptional status of cells and proteomic studies of the cell nucleus.

## References

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## Related Sigma Products

Product Name	Product Number
Nuclei EZ Prep™ nuclei isolation kit	NUC-101
Nuclei PURE Prep™ nuclei isolation kit	NUC-201
NuCLEAR™ extract kit	N-XTRACT
Mobility Shift Optimization kit	SHIFT-1
Protease Inhibitor Cocktail	P 8340
Ribonuclease Inhibitor (from human placenta)	R 7253