

Isolation and Use of Mammalian Cell Nuclei

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Introduction

Fractionation of cells into their subcellular components has long been a central approach in cell biology. Subcellular fractionation techniques have been used widely to study structure and function of organelles and subcellular compartments, as well as to examine the location, processing, and trafficking of molecular components.¹ The object of most subcellular fractionation procedures is to obtain cellular organelles and macromolecules in a functional state, in which they retain most of their original biochemical properties. This is usually achieved by employing cell lysis by gentle mechanical means or with mild detergents, followed by fractionation of cellular components by differential centrifugation.^{2,3}

The study of the cell nucleus and nuclear events has been necessary for understanding a number of processes of primary importance in cell biology, including chromatin structure, transcriptional regulation of gene expression, RNA synthesis and processing, mechanism and regulation of bi-directional nuclear transport, and nuclear apoptosis. Since preparation of nuclei or nuclear extracts is often the first step in studying nuclear components and events, a number of different techniques for isolating nuclei have been described. These methods vary considerably, depending on species or tissue type and the downstream application for the isolated nuclei. This review describes the most widely used methods for isolation and use of mammalian cell nuclei, and introduces several new products that incorporate improvements to standard methods.

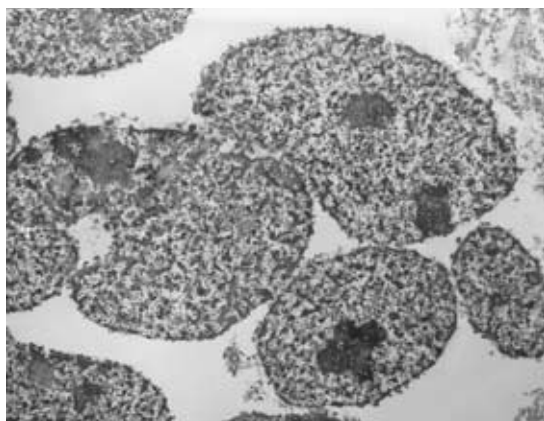
Discussion

It is important to choose carefully a method of nuclei isolation that will yield nuclei with the desired properties. In some cases, such as investigations of nuclear transport and *in vitro* studies of nuclei assembly and disassembly

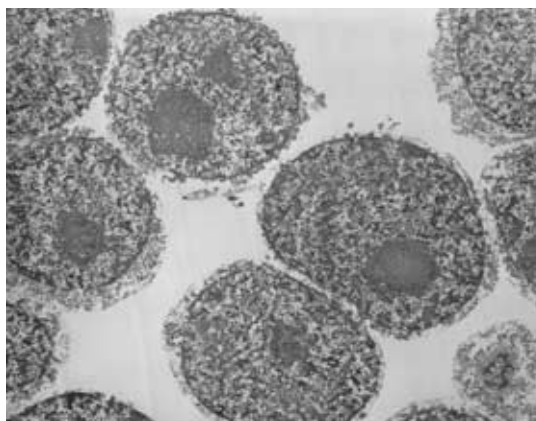
processes, it is necessary to isolate nuclei with intact nuclear envelopes.⁴ In these cases the isolated nuclei have intact inner and outer membranes and nuclear pore structures. Since the outer nuclear membrane is continuous with the rough endoplasmic reticulum (RER) of the cytoplasm, these nuclei can have significant cytoplasmic contamination that can interfere with purification of nuclear components or obscure the proper interpretation of molecular localization studies. In addition, the nuclear membrane is a selective barrier, which can compromise rapid and efficient entry of small molecules, such as nucleotide precursors used for labeling nascent RNA chains in transcription run-off experiments. Therefore, procedures which remove the nuclear membranes, but yield otherwise intact nuclei, are frequently preferred for the study of nucleoplasmic components and functions other than nuclear transport.

There are several widely accepted standard methods for isolating nuclei from mammalian cells⁵⁻⁷ that employ gentle, non-ionic detergents and yield nuclei free from RER and other cytoplasmic contamination. The resulting nuclei are functional for the synthesis and extension of endogenous RNA primary transcripts. The method for nuclei isolation from tissue culture cells utilizes a hypotonic Nonidet P-40 or Igepal CA-360 detergent lysis buffer.^{6,7} Since nuclei are the largest organelles in the cell, they are easily separated from other organelles and detergent-soluble contaminants by low speed centrifugation and further purified by repeated washes in the same lysis buffer. For isolation of nuclei from solid tissues or from cell lines with fragile nuclei, the nuclei are purified by centrifugation through a dense sucrose cushion to protect nuclei and strip away cytoplasmic contaminants.⁵⁻⁷

We have recently improved the method for isolation of nuclei from adherent tissue culture cell lines. In the standard protocol^{6,7} cells are harvested by scraping in phosphate buffered saline (PBS) and collected by centrifugation, before being lysed in the detergent containing lysis buffer. However, scraping cells in PBS yields a heterogeneous population of intact cells, physically damaged cells, cell debris, and free nuclei. In addition, the harvest of adherent cells by scraping in PBS is inefficient. It often results in low and variable yields of nuclei, and delay in time before all the cells are lysed. In the improved method (Nuclei EZ Prep Nuclei Isolation Kit,



**HEK 293
(adherent)**

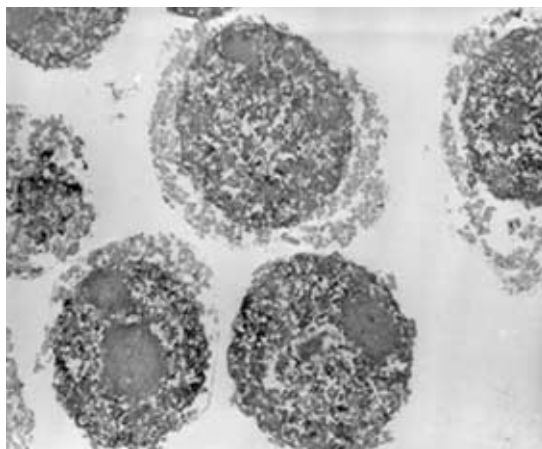


**Jurkat
(suspension)**

Figure 1. Electron micrographs of purified nuclei. Nuclei isolated by the Nuclei EZ Prep nuclei isolation kit were fixed, sectioned, stained, and visualized by transmission EM.

Product Code: NUC-101), cells are harvested and lysed simultaneously. This modification is critical for rapid, efficient and complete lysis of the cells, and decreases the possibility of artifacts due to the cell harvesting procedure. The isolated nuclei appeared to be structurally intact and free from RER and cytoplasmic debris (Figure 1).

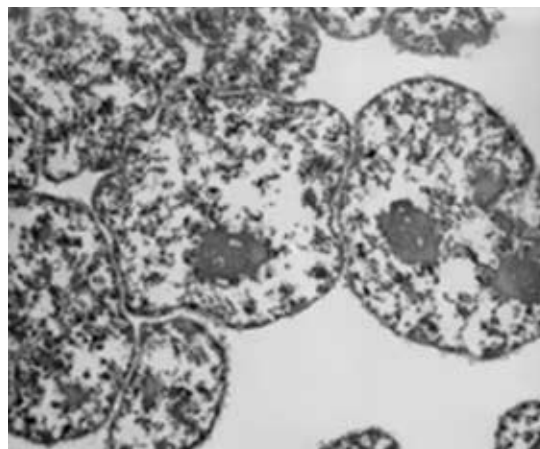
For nuclei isolation from animal tissues, from cultured cells with fragile nuclei, or from cultured cells that are difficult



**Jurkat
(suspension)**

Optimization Kit, Product Code: SHIFT-1) and for *in vitro* transcription reactions.

All nuclei isolation procedures have some common potential technical problems. Contamination by endogenous proteases or nucleases, and extraction or physical perturbations by detergents may cause adverse effects on the quality of the nuclei. The nuclei may become more fragile due to the loss of nuclear



**Rat Liver
(solid tissue)**

Figure 2. Electron micrographs of nuclei purified with 1.8 M sucrose cushions. Nuclei isolated by the Nuclei PURE Prep nuclei isolation kit were fixed, sectioned, stained, and visualized by transmission EM.

to harvest or lyse (e.g., epithelial-like cells with tight junctions), the preferred method remains detergent and physical lysis by homogenization. This is done in an iso-osmotic sucrose buffer with subsequent purification of the nuclei by ultracentrifugation through a dense (2 M) sucrose cushion.⁵⁻⁷ However, many mammalian nuclei are not dense enough to pass through a 2 M sucrose cushion, resulting in poor yields of purified nuclei. We recently improved the standard protocol by decreasing the sucrose cushion concentration from 2 M to 1.8 M sucrose (Nuclei PURE Prep Nuclei Isolation Kit, Product Code: NUC-201). Nuclei from a variety of mammalian cells passed through the 1.8 M sucrose cushion, resulting in greatly improved yields. The 1.8 M sucrose cushion purified nuclei appeared to be structurally intact and free from RER and cytoplasmic debris (Figure 2).

In addition to high yields of pure nuclei (Figures 1 and 2), the resulting nuclei from both of the improved methods are also functional for run-off synthesis of RNA polymerase II dependent RNA transcripts (Figure 3). Therefore, these procedures should be useful for examining the transcriptional state of mammalian cells in nuclear transcription run-off experiments.

Also, nuclei can be isolated and nuclear extracts can be prepared in one integrated protocol (Nu-CLEAR™ Extraction Kit, Product Code: N-XTRACT). Such nuclear extracts are useful for analyzing transcription factors by Electrophoretic Mobility Shift Assays (Mobility Shift

membranes and, consequently, the purified nuclei may aggregate if excessive DNA leakage occurs. These potential problems are usually overcome by rapidly isolating the nuclei from fresh cells or tissue and by keeping the nuclei cold (4 °C) during the isolation procedure. If necessary, protease and/or nuclease inhibitors may be added to the lysis buffers to minimize enzymatic hydrolysis of the molecules of interest.

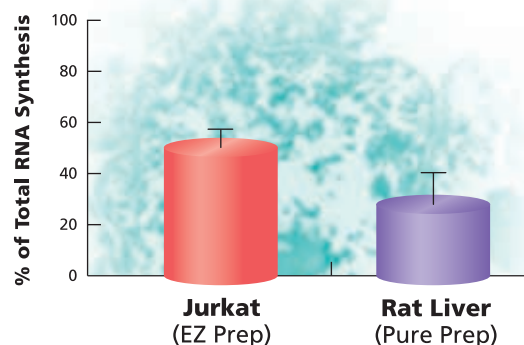


Figure 3. In vitro mRNA synthesis by nuclei. Nuclei were isolated and labeled with [32 P]GTP. Synthesis of mRNA was determined as the percentage difference between incorporation in the absence and presence of 0.25 μ g/ml 32 P-amanitin. Error bars represent standard deviation.

Summary

Methods of nuclei isolation will continue to be useful for many common cell biology applications, such as purification of nuclear components (chromatin, genomic DNA, histones and nuclear RNA/RNP) and for functional studies, such as examination of the transcriptional status of cells by *in vitro* transcription run-off analysis.⁵⁻⁷ Improved techniques, such as those reported here, will be increasingly useful for isolation of nuclei for a variety of applications, including newer methods such as *in vitro* nuclear apoptosis assays,^{8,9} and transcription profiling. In addition, these and other cellular fractionation methods will be important in the future for the emerging areas of functional genomics and proteomics.¹⁰

References

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ORDERING INFORMATION

Product Code	Product Name	Unit	Price
NUC-101	Nuclei EZ Prep nuclei isolation kit	1 kit (25 nuclei preps)	\$98.50
NUC-201	Nuclei PURE Prep nuclei isolation kit	1 kit (15 nuclei preps)	\$203.00

RELATED PRODUCTS

Product Code	Product Name	Unit	Price
N-XTRACT	Nu-CLEAR™ extraction kit	1 kit (100 preps)	\$182.70
SHIFT-1	Mobility Shift Optimization kit	1 kit (100 trials)	\$195.30
P 8340	Protease Inhibitor Cocktail	1 ml 5 ml	\$28.20 \$113.00
R 7253	Ribonuclease Inhibitor from human placenta	300 units 1500 units 5000 units 30,000 units	\$17.60 \$57.30 \$223.70 \$654.05