INSTRUCTIONS

Nuclei Enrichment Kit for Tissue

PIERCE
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89841

1847.0

Number	Description
89841	**Nuclei Enrichment Kit for Tissue**, contains sufficient reagents to perform 25 enrichments of intact nuclei from soft and hard tissue
	Kit Contents:
	Nuclei Enrichment Reagent A, 90 ml
	Nuclei Enrichment Reagent B, 90 ml
	OptiPrep™ Cell Separation Media, 50 ml
	BupH™ Phosphate Buffered Saline, 1 pack, results in 0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7.2 when reconstituted with 500 ml of ultrapure water
	Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Nuclei Enrichment Kit for Tissue enables isolation of an enriched sample containing intact nuclei from 400-500 mg of either hard or soft tissue. The kit contains OptiPrep™ Cell Separation Media and optimized buffers along with detailed instructions for the enrichment of nuclei with minimal contamination from golgi bodies and endoplasmic reticulum. The procedure provided is a guideline and some optimization may be required to ensure optimal results for each tissue type. The isolated, intact nuclear sample contains all the nuclear proteins, genetic material and other intact nuclear structures, allowing downstream applications such as enzymatic assays, electron microscopy, Western blotting and 2D/MS analysis.

Important Product Information

- This kit contains sufficient reagents for 25 isolations of intact nuclei from 400-500 mg of tissue. This procedure is not for cultured cells.
- The OptiPrep™ Cell Separation Media is a 60% stock solution of iodixanol – 5,5′-[(2-hydroxy-1,3-propanediyl)-bis(acetylamino)] bis [*N,N*′-bis(2,3 dihydroxypropyl-2,4,6-triiodo-1,3-benzenecarboxamide).
- Optimization of the density gradients may be required for each type of tissue. Refer to the Axis-Shield website for helpful density gradient concentrations for various tissue types: www.axis-shield.com
- This procedure was optimized for isolating nuclei from rat kidney and liver tissues with minimal contamination from other organelles.
- Starve animals 24 hours before sacrifice to minimize lipid interference.
- This procedure is for use with a tabletop ultracentrifuge. Appropriately scale reagents when using a floor-model ultracentrifuge.

Additional Materials Required

- Ultracentrifuge: tabletop or floor-model, capable of reaching a speed of 40,000 × *g* at 2-8°C.
- Ultracentrifuge tubes: either open- or closed-top, 8 ml
- Tissue tearer, for example, Kinematica AG Polytron® PT1200
- 2 ml microcentrifuge tubes
- Protease inhibitors, EDTA-free such as Halt™ Protease Inhibitor Cocktail Kit (Product No. 78415)
- Bench-top microcentrifuge with refrigeration
- 12 × 75 mm glass test tubes
- 15 ml conical tubes
- Vortex mixer
- Surgical scissors for mincing tissue

Protocol for the Enrichment of Nuclei from Soft and Hard Tissue

A. Material Preparation

Phosphate-buffered Saline (PBS)	Dissolve the dry-blend buffer with 500 ml of ultrapure water. For long-term storage of excess buffer, sterile-filter the solution and store at 4°C.
Gradient Dilution Buffer	Mix equal volumes of Nuclei Enrichment Reagents A and B. Each sample requires 3.20 ml of Gradient Dilution Buffer.
OptiPrep™ Gradients	The OptiPrep™ Media is supplied as a 60% solution. Prepare three gradient solutions using the Gradient Dilution Buffer as indicated in the table below. The final volume is sufficient for one sample preparation.

Mix			
OptiPrep Media Volume (µl)	**Gradient Dilution Buffer Volume (µl)**	**Final Volume (µl)**	**Gradient %**
690	810	1,500	27.5
575	925	1,500	23
330	675	1,000	20

B. Soft or Hard Tissue Preparation

1. Immediately before use, add protease inhibitors to the Nuclei Enrichment Reagents A and B; add inhibitors only to the volume being used for the procedure and not to the stock solution.
2. Wash 400-500 mg of tissue twice with 2-4 ml of PBS. Carefully remove and discard the PBS wash.
3. Transfer tissue to a new 12 × 75 mm glass test tube, cut tissue into small pieces (< 3 mm^3) and add 800 µl of Reagent A and 800 µl of Reagent B.

   Note: Ensure that all tissue is immersed in Reagents A and B before homogenizing.
4. Using a tissue tearer, homogenize the tissue for 30 seconds at 8,000 RPM while on ice.
5. Transfer the homogenate to a new 2 ml microcentrifuge tube.
6. Close the tube and invert five times to ensure that all reagents are mixed – do not vortex.
7. Centrifuge tube at 500 × g for 10 minutes at 4°C.
8. Transfer the supernatant to a new 15 ml conical tube and discard the pellet.

   Note: If lipids are present after centrifugation, use a pipette to remove the lipids from the top of the sample before transferring the supernatant to a new tube.

C. Density Gradient Centrifugation

Note: The density gradient concentrations may require optimization, depending on tissue type.

Note: An open- or closed-top ultracentrifuge tube can be used for isolation.

1. Prepare a discontinuous density gradient by carefully overlaying the three OptiPrep™ Gradients (see Section A) in an ultracentrifugation tube. Add the highest concentration (27.5%) first.
2. Mix the prepared tissue extract with OptiPrep™ Cell Separation Media (60%) and the Gradient Dilution Buffer to make a final density gradient of 7.5%.

Tissue Extract Volume (µl)	OptiPrep™ Media Volume (µl)	Gradient Dilution Buffer Volume (µl)	OptiPrep™ Media Final Concentration
1,600	300	800	7.5%

3. Overlay the sample containing the 7.5% OptiPrep™ Media on top of the density gradients.
4. Ultracentrifuge the sample at 40,000 × *g* for 90 minutes at 4°C.
5. Use a pipette to transfer the top 2.0 ml, which contains the nuclei, into a 15 ml conical tube. Discard the remainder of the sample.

   Note: If lipids are present at the top of the tube, avoid pipetting them into the sample.

Troubleshooting

Problem	Possible Cause	Solution
Low enrichment of nuclei	Contaminants in the nuclear fraction after ultracentrifugation	After the 500 × *g* centrifugation, pipette off as many lipids as possible
	Poor lysis of tissue sample	Homogenize longer, but monitor lysis so as to not damage the organelle
Large amount of lipids observed near the nuclear rich fraction after ultracentrifugation	Large amount of lipids present in the sample before mixing with OptiPrep™ Gradients	Starve animal for 24 hours before sacrifice
		After the 500 × *g* centrifugation, pipette off as many lipids as possible

Additional Information

Optimization of OptiPrep™ Cell Separation Media Concentration

The density of nuclei varies depending on tissue type. The optimum concentration for effective nuclei isolation must be determined empirically. See the Axis-Shield website (http://www.axis-shield.com) for examples of various OptiPrep™ Media concentrations with different tissue types. Post-ultracentrifugation, collect small volumes of the sample/gradient, such as 500-1,000 µl fractions, from the top of the gradient going down and examine the location of the nuclei and the contaminating organelles via Western blot.

Related Pierce Products

23226	**Coomassie Plus – The Better Bradford™ Assay Kit**
78415	**Halt™ Protease Inhibitor Cocktail, EDTA-Free,** 1 ml
78833	**NE-PER® Nuclear and Cytoplasmic Extraction Kit**
89840	**Peroxisome Enrichment Kit for Tissue**
89839	**Lysosome Enrichment Kit for Tissue and Cultured Cells**
89801	**Mitochondria Isolation Kit for Tissue**
89874	**Mitochondria Isolation Kit for Cultured Cells**