INSTRUCTIONS

Peroxisome Enrichment Kit for Tissue

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89840

1846.0

Number	Description
89840	**Peroxisome Enrichment Kit for Tissue**, sufficient reagents to perform 25 enrichments of intact peroxisomes from 50-300 mg of soft or hard tissue

Kit Contents:

Peroxisome Enrichment Reagent A, 90 ml

Peroxisome 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 ultrapure water

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Table of Contents

Introduction

The Peroxisome Enrichment Kit for Tissue enables isolation of an enriched peroxisome sample from hard or soft tissue such as rat liver, kidney and heart tissue. The kit provides sufficient reagents for preparing 25 extracts and uses OptiPrep™ Cell Separation Media for the density-based separation of peroxisomes with minimal contamination from golgi bodies and endoplasmic reticulum. The isolated peroxisomes can be used for many downstream applications, including 2D/MS, electron microscopy, enzymatic assays and Western blotting.

Important Product Information

- This kit contains sufficient reagents for 25 isolations of intact peroxisomes from 50-300 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 peroxisomes 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.
- Peroxisomes are isolated in OptiPrep™ Media, which is suitable for many downstream applications; if required, peroxisomes can be pelleted by centrifuging at 18,000 × *g*.

Additional Materials Required

- Tabletop or floor-model ultracentrifuge capable of 180,000 × *g* at 2-8°C
- 8 ml ultracentrifuge open- or closed-top tubes able to withstand 180,000 × *g*
- Tissue tearer, for example, Kinematica AG Polytron® PT1200
- 2.0 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
- Surgical scissors for mincing tissue

Procedure for Enrichment of Peroxisomes from Soft Tissue

A. Soft Tissue 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 the Peroxisome Enrichment Buffers A and B. Each sample requires 1.88 ml of Gradient Dilution Buffer.

OptiPrep™ Gradients: The OptiPrep™ Media is supplied as a 60% solution. Prepare two 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 %
1,170	830	2,000	35
750	750	1,500	30

B. Soft Tissue Preparation

1. Immediately before use, add protease inhibitors to Peroxisome Enrichment Reagents A and B; add inhibitors only to the volume being used for the procedure and not to the stock solution.
2. Wash 50-300 mg of soft tissue twice with 2-4 ml of PBS. Carefully remove and discard the PBS wash.
3. Transfer tissue to a 12 × 75 mm glass test tube, cut tissue into small pieces (< 3 mm^3) and add 800 µl of Peroxisome Enrichment Reagent A.

   Note: Ensure that all tissue is immersed in Reagent A before homogenizing.
4. Using a tissue homogenizer, homogenize the tissue for 30 seconds at 8,000 RPM while on ice.
5. Transfer the homogenate to a 2.0 ml microcentrifuge tube and add 800 µl of Peroxisome Enrichment Reagent B. Invert the tube 15 times to mix – do not vortex.
6. Centrifuge tube at 500 × *g* for 10 minutes at 4°C.
7. Transfer the supernatant to a new 2.0 ml microcentrifuge 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.
8. Centrifuge the supernatant at 1,000 × *g* for 10 minutes at 4°C.

9. Transfer the supernatant to a 15 ml conical tube and discard the pellet. Keep sample on ice until needed.

   Note: If lipids are present after centrifugation, remove them before transferring the supernatant to a new tube.

C. Soft Tissue Density Gradient Centrifugation

- The concentrations of the density media for peroxisome isolation may require optimization depending on tissue type. Refer to the references for examples of gradient concentrations.

1. Prepare a discontinuous density gradient by carefully overlaying the two OptiPrep™ Gradients (see Section A. Soft Tissue Material Preparation) in an ultracentrifuge tube. Add the highest concentration (35%) first.
2. Mix the tissue extract prepared from Section B with the OptiPrep™ Cell Separation Media and Gradient Dilution Buffer to make a final concentration of 27.5%, as indicated in the table below.

Tissue Extract Volume (µl)	60% OptiPrep™ Media Volume (µl)	Gradient Dilution Buffer Volume (µl)	OptiPrep™ Media Final Concentration
1,600	1,350	300	27.5%

3. Overlay the sample containing the 27.5% OptiPrep™ Media on top of the density gradients. Ultracentrifuge the tube at 180,000 × *g* for 90 minutes at 4°C.

   Note: A visible white band, which contains the peroxisomes, forms near the bottom of the centrifuge tube.
4. Carefully pipette ~3.5 ml from the top of the centrifuge tube and discard. To minimize sample contamination and peroxisome loss, do not disrupt the white band.
5. Use a 1,000 µl pipette to carefully remove the next 2.0 ml, which contains the peroxisomes, and place into a 15 ml conical tube. Keep peroxisomes on ice or store at -20°C.

D. Optional Removal of Peroxisomes from OptiPrep™ Cell Separation Media

1. Add 3.0 ml of PBS to the tube containing the 2.0 ml of isolated and enriched peroxisomes. Vortex the tube on high for 15 seconds.
2. Centrifuge the peroxisomes at 18,000 × *g* for 20 minutes.
3. Discard the supernatant. Resuspend the peroxisome pellet in a buffer compatible with the subsequent downstream application. Keep peroxisomes on ice or store at -20°C.

Procedure for Enrichment of Peroxisomes from Hard Tissue

A. Hard Tissue 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 the Peroxisome Enrichment Buffers A and B. Each sample requires 2.6 ml of Gradient Dilution Buffer
OptiPrep™ Gradients	The OptiPrep™ Media is supplied as a 60% solution. Prepare two 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)	Gradient Volume (µl)	% Gradient Dilution
920	1,100	2,000	27.5%
500	1,000	1,500	20%

B. Hard Tissue Preparation

1. Immediately before use, add protease inhibitors to Peroxisome Enrichment Reagents A and B; only add inhibitors to the volume being used for the procedure and not to the stock solution.
2. Wash 50-200 mg of hard tissue twice with 2-4 ml of PBS. Carefully remove and discard the PBS wash.
3. Transfer tissue to a 12 × 75 mm glass test tube, cut tissue into small pieces (< 2 mm^3) and add 800 µl of Peroxisome Enrichment Reagent A.

   Note: Ensure that all tissue is immersed in Reagent A before homogenization.
4. Using a tissue homogenizer, homogenize the tissue for 30 seconds at 8,000 RPM while on ice.
5. Transfer the homogenate to a 2.0 ml microcentrifuge tube, and add 800 µl of Peroxisome Enrichment Reagent B to the homogenate. Invert tube 15 times to mix – do not vortex.
6. Centrifuge at 500 × *g* for 10 minutes at 4°C.
7. Transfer the supernatant to a 2.0 ml microcentrifuge 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.
8. Centrifuge the supernatant at 1000 × *g* for 10 minutes at 4°C.
9. Transfer the supernatant to a 15 ml conical tube and discard the pellet. Keep sample on ice until needed.

   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. Hard Tissue Density Gradient Centrifugation

1. The concentrations of the density media for peroxisome isolation may require optimization, depending on tissue type. (Refer to references for examples of gradient concentrations.)
2. Prepare a discontinuous density gradient by carefully overlaying the two OptiPrep™ Gradients (see Section A. Hard Tissue Material Preparation) in an ultracentrifuge tube. Add the highest concentration (27.5%) first.
3. Mix the prepared hard tissue extract with the OptiPrep™ Cell Separation Media (60%) and Gradient Dilution Buffer to make a final concentration of 18%.

Tissue Extract Volume (µl)	60% OptiPrep Media Volume (µl)	Gradient Dilution Buffer Volume (µl)	OptiPrep™ Media Final Concentration
1,600	900	500	18%

4. Overlay the sample containing the 18% OptiPrep™ Media on top of the density gradients
5. Ultracentrifuge at 180,000 × *g* for 90 minutes at 4°C.

   Note: A visible white band, which contains the peroxisomes, forms near the bottom of the centrifuge tube.
6. Carefully pipette ~3.5 ml off the top of the centrifuge tube and discard. To minimize sample contamination and peroxisome loss, do not disrupt the white band.
7. Use a 1,000 µl pipette to carefully remove the next 2.0 ml, which contains the peroxisomes, and place into a 15 ml conical tube. Keep peroxisomes on ice or store at -20°C.

D. Optional Removal of Peroxisomes from OptiPrep™ Cell Separation Media

1. Add 3.0 ml of PBS to the tube containing the 2.0 ml of isolated and enriched peroxisomes. Vortex the tube on high for 15 seconds.
2. Centrifuge the peroxisomes at 18,000 × *g* for 20 minutes.
3. Remove the supernatant and discard. Resuspend the peroxisome pellet in a buffer compatible with the downstream application. Keep peroxisomes on ice or store at -20°C.

Troubleshooting

Problem	Possible Cause	Solution
Low enrichment	Insufficient tissue lysis	Homogenize longer, being careful to avoid damaging the peroxisomes
	Large amount of lipids present in the sample caused the peroxisomes to stay in the low % gradient	Pipette lipids from the top of the supernatant after the 500 and 1,000 × *g* centrifugations
Contaminating organelles	For hard tissue, mitochondria co-purifies with the peroxisomes because of its overlapping density	None
	Gradients were mixed while they were being layered on top of one another	Be extremely careful when adding each gradient to the centrifuge tube
		Siphon the media carefully so as not to disrupt the other gradients

Additional Information

A. Alternative Lysis Method

Dounce homogenization can be used for lysis instead of a tissue tearer for peroxisome enrichment. The number of Dounce strokes required for optimum cell lysis and minimal damage to the peroxisomes requires optimization for each sample type. Use the table below as a guide.

Tissue Type	Amount (mg)	Number of Dounce Strokes
Soft (Liver)	50	20-30
	300	60-85
Hard (Heart)	50	10
	200	30-50

* For hard tissue, mince the tissue into smaller pieces before homogenization.

B. Optimization of OptiPrep™ Cell Separation Media Concentration

The densities of peroxisomes vary with different tissue types. The optimum concentration for effective peroxisome isolation and enrichment will need to be determined by the user. See references and Axis-Shield website (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 peroxisomes 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

89841 **Nuclei 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**

References

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