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OrgFrontier™ Peroxisome Isolation Kit

11/19

(Catalog # K2027-20; 20 preparations; Store at Multiple Temperatures)

I. Introduction:

Peroxisomes are small, single membrane-bound organelles that are found in all eukaryotic cells and are similar to lysosomes in size and morphology. Peroxisomes contain a variety of enzymes that utilize molecular oxygen to perform oxidation reactions. Peroxisomal enzymes are involved in the oxidation of ethanol, long-chain fatty acids, branched chain fatty acids, D-amino acids, and polyamines. Hydrogen peroxide, a product of the oxidative enzymatic reaction is subsequently converted to water and oxygen by catalase, an abundant peroxisomal enzyme. Peroxisomes are also involved in the biosynthesis of ether phospholipids known as plasmalogens that are required for the myelination of neurons. **BioVision's OrgFrontier™ Peroxisome Isolation Kit** provides a proprietary set of reagents and buffers designed to enable the extraction and isolation of intact peroxisomes from mammalian tissues and cultured cells. The protocol requires an ultracentrifugation step and can be completed in one day.

II. Applications:

- Isolation of peroxisomes from mammalian tissues and cultured cells.
- Peroxisome studies and peroxisome protein profiling.
- Enrichment of peroxisome-associated proteins for western blot and ELISA.

III. Sample Type:

- Cultured mammalian cells and mammalian tissues

IV. Kit Contents:

Components	K2027-20	Cap Code	Part Number
Homogenization Buffer	100 ml	Red/NM	K2027-20-1
EZBlock™ Protease Inhibitor Cocktail	1 ml	Orange	K2027-20-2
Gradient Dilution Buffer	110 ml	NM	K2027-20-3
OptiPrep™ Density Gradient Medium	90 ml	Blue/NM	K2027-20-4

V. User Supplied Reagents and Equipment:

- Centrifuge (refrigerated)
- Microcentrifuge tubes
- Ultracentrifuge (capable of 180,000 x *g*)
- Compatible (~10 ml) ultracentrifuge tubes
- Dounce Homogenizer (BioVision Cat # 1998)
- Scalpel or Scissors
- 1X PBS

VI. Storage Conditions and Reagent Preparation:

Store kit components at -20°C except for OptiPrep™ Density Gradient Medium, which should be stored at 4°C upon receipt. Protect from light. Read the entire protocol before performing the assay.

- **Homogenization Buffer & Gradient Dilution Buffer:** Thaw completely and chill on ice prior to use. Store at -20°C or 4°C.
- **EZBlock™ Protease Inhibitor Cocktail:** Thaw to room temperature (RT) prior to use.
- **OptiPrep™ Density Gradient Medium:** Thaw completely and store at 4°C. Mix thoroughly before use.
- **Working Buffer solution:** Add 10 µl of Protease Inhibitor Cocktail to 5 ml of Homogenization Buffer to prepare the **Working Buffer (WB)** solution prior to use. Keep on ice and discard any unused solution after 8 hr.
- **Gradient Working solution (GW, 54%):** Prepare 5 ml Gradient Working **(GW)** solution (equivalent to one isolation) by mixing 4.5 ml of OptiPrep™ Density Gradient Medium with 0.5 ml of Gradient Dilution Buffer. Keep the GW solution on ice at all times.
- **36% Gradient Solution:** Prepare 3 ml of 36% Gradient Solution (equivalent to one isolation) by mixing 2.01 ml of GW solution with 0.99 ml of Gradient Dilution Buffer. Keep the 36% Gradient Solution on ice at all times.
- **27.5% Gradient Solution:** Prepare 2 ml of 27.5% Gradient Solution (equivalent to one isolation) by mixing 1.02 ml GW solution with 0.98 ml of Gradient Dilution Buffer. Keep the 27.5% Gradient Solution on ice at all times.
- **22.5% Gradient Solution:** Prepare 2 ml of 22.5% Gradient Solution (equivalent to one isolation) by mixing 0.836 ml GW solution with 1.17 ml of Gradient Dilution Buffer. Keep the 22.5% Gradient Solution on ice at all times.

VII. Peroxisome Isolation Protocol:

1. Sample Preparation:

a. Cultured cells: To prepare cell homogenates, start with 2-5 x 10^8 cells. Collect suspension cells by centrifugation (300 x *g* and 4°C for 5 min). For adherent cells, aspirate the growth medium and wash cells with 1X PBS. Scrape off the cells and pellet by centrifugation at 300 x *g* and 4°C for 5 min. Resuspend the cell pellet in 2 ml of ice-cold Working Buffer Solution. Transfer the cell suspension to a pre-chilled Dounce Homogenizer (BioVision Cat # 1998) and homogenize the cells on ice using a tight fitting homogenizer pestle for ~ 50-100 strokes.

b. Mammalian tissues: Begin with ~0.3-0.7 g of wet tissue. Mince tissue into small pieces using clean scissors or scalpel. Rinse tissue pieces with ice-cold 1X PBS. Gently blot the tissue to remove the excess moisture and transfer the tissue sample to a pre-chilled Dounce Homogenizer (BioVision Cat # 1998). Add 2 ml of ice-cold Working Buffer solution and homogenize the tissues on ice using a tight fitting homogenizer pestle for ~ 50-100 strokes.

Note: Efficient homogenization depends on the cell or tissue type. To check the efficiency of the homogenization, mix 2-3 µl of the homogenized suspension with an equal volume of Trypan Blue solution. Pipet few drop of the homogenized suspension on a slide or hemacytometer and observe it under the microscope. Look for viable cells. A "shiny ring" around the nuclei indicates that cells are still intact. If 70-80% of the nuclei do not have the shiny ring, proceed to the next step. Otherwise, perform an additional 10-30 stroke.

2. Peroxisome Isolation:

a. Transfer the homogenate to a clean, pre-chilled microcentrifuge tube.

b. Centrifuge at 1000 x *g* for 10 min at 4°C. Carefully remove and discard any fatty residue that is formed at the top of the supernatant. Collect the remaining supernatant and transfer it to a new pre-chilled tube. Store the supernatant on ice.

c. Resuspend the pellet from step 2b in 1 ml of Working Buffer solution. Homogenize the resuspended pellet in Dounce Homogenizer for ~ 25-50 strokes and centrifuge at 1000 x *g* for 10 min at 4°C.

d. Pool the supernatant fractions. ***This is the post-nuclear fraction.*** Discard the pellet.

e. Centrifuge the supernatant at 5000 x *g* for 10 min at 4°C. Carefully remove and discard any fatty residue formed at the top of the Supernatant. Collect the remaining supernatant and transfer it to a new pre-chilled microcentrifuge tube. Store the supernatant on ice. ***This step will remove the remaining nuclei and most of the mitochondria.*** Discard the pellet.

f. Centrifuge the supernatant 20,000 x *g* for 20 min at 4°C. Remove and discard the Supernatant. The pellet is the light mitochondrial fraction (LMF) and contains lysosomes, peroxisomes and any remaining mitochondria.

Note: Perform all steps on ice or at 4°C.

3. Ultracentrifugation and Fraction Collection:

a. Resuspend the LMF pellet in 1.5 ml Gradient Dilution Buffer.

b. Homogenize the LMF pellet mixture using a **loose fitting homogenizer pestle** for 10-15 strokes.

c. Remove the homogenized sample. Measure the actual volume and mix with an equal volume of the 36% Gradient Solution. This is the 18% LMF solution.

d. Place 2 ml of the 27.5% Gradient Solution on the bottom of a fresh, clean, pre-chilled 10 ml ultracentrifuge tube (**see note**). Carefully place 2 ml of the 22.5% Gradient Solution on top of the 27.5% Gradient Solution. *Do not mix or perturb the layers*.

e. Finally, layer the 18% LMF solution carefully on top of the 22.5% Gradient Solution. *Do not mix or perturb the layers*.

f. Centrifuge the tube(s) in a ultracentrifuge at 180,000 x *g* for 3 hr at 4°C. ***Program the centrifuge so that there is no braking at the end of the run.***

g. At the end of the ultracentrifugation, the peroxisomal fraction(s) will be in the visible band, at the interface of the 22.5%/27.5% Gradient Solutions. There may also be some peroxisomes floating in the bottom layer.

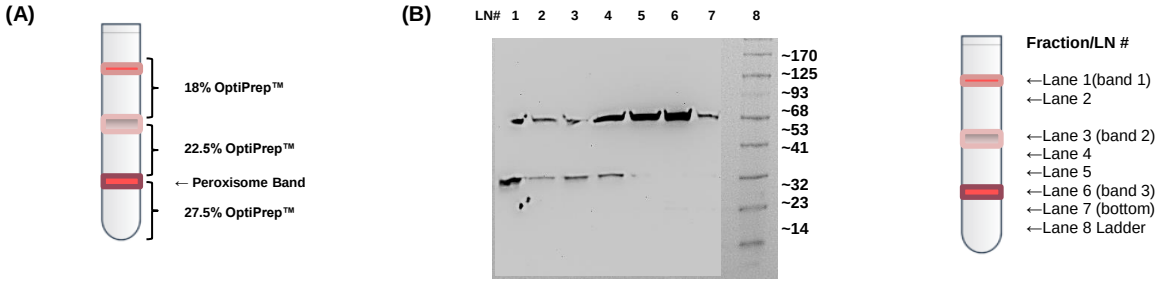
h. Carefully collect the band at the 22.5%/27.5% layer interface and if desired, the bottom layer in a separate, ice-cold tube. These are the isolated peroxisomes.

i. The peroxisomes can be washed by pelleting the fraction(s) at 20,000 x *g* and resuspending in your buffer of choice.

Notes:

a. This procedure uses 10 ml ultracentrifuge tubes.

b. Larger or smaller tubes can be used as long as the final OptiPrep™ Gradient Solution ratios remain the same.



Figures: (A) Representation of an ultracentrifuge tube using OptiPrep™ Density Gradient Medium from step h. under section 3. Following ultracentrifugation, the isolated peroxisomal fraction is localized at the interface between the 22.5% and 27.5% density layers (denoted by the red shaded line). **(B)** Western Blot showing the Peroxisomal fraction using Catalase antibody (BioVision Cat# 3806), MW ~ 60kDa and Mitochondrial fraction using VDAC antibody (BioVision Cat# 3594), MW ~30kDa respectively.

VIII. Related Products:

Nuclear/Cytosol Fractionation Kit (K266)
Catalase Activity Colorimetric/Fluorometric Assay Kit (K773)
BCA Protein Assay Kit (K818)
Lysosome Purification Kit (K235)
Plasma Membrane Isolation Kit (K414)
Plasma Membrane Protein Extraction Kit (K268)
Uricase Activity Assay Kit (K734)
OrgFrontier™ Chloroplast Isolation Kit (K468)
Microsome Isolation Kit (K249)
Mammalian Mitochondria Isolation Kit (K288)

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