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Product Information

Microsome Isolation Kit

Catalog Number **MAK340**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Microsomes are spherical vesicle-like structures formed from membrane fragments following homogenization and fractionation of eukaryotic cells. The microsomal subcellular fraction is prepared by differential centrifugation and consists primarily of membranes derived from the endoplasmic reticulum (ER) and Golgi apparatus.

Microsomes isolated from liver tissue are used extensively in pharmaceutical development, toxicology, and environmental science to study the metabolism of drugs, organic pollutants, and other xenobiotic compounds by the cytochrome P450 monooxidase (CYP) enzyme superfamily. Microsomal preparations are an affordable and convenient *in vitro* system for assessing Phase I biotransformation reactions, as they contain all of the xenobiotic-metabolizing CYP isozymes and the membrane-bound flavoenzymes (such as NADPH P450-Reductase and cytochrome b_5) required for function of the multicomponent P450 enzyme system.

The Microsome Isolation Kit enables preparation of active microsomes in about one hour, without the need for ultracentrifugation or sucrose gradient fractionation. The kit contains sufficient reagents for 50 isolation procedures, yielding microsomes from roughly 25 grams of tissue or cultured cells.

This kit is suitable for the isolation of microsomes from mammalian glands and soft tissues such as liver, spleen, lungs, etc. or cultured eukaryotic cell lines such as HepG2 human hepatic carcinoma cells.

Components

The kit is sufficient for 50 isolations.

Homogenization Buffer Catalog Number MAK340A	80 mL
Storage Buffer Catalog Number MAK340B	20 mL
Protease Inhibitor Cocktail Catalog Number MAK340C	1 Vial

Reagents and Equipment Required but Not Provided.

- Refrigerated microcentrifuge capable of RCF ≥20,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Anhydrous dimethyl sulfoxide (DMSO) (Catalog Number 276855)
- Pasteur pipettes
- Phosphate Buffered Saline (Catalog Number P3813)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C upon receiving. The Homogenization and Storage Buffers may be stored at either 2–8 °C or –20 °C. Keep buffers on ice while in use.

Preparation Instructions

This procedure is designed for isolation of microsomes from up to 400 mg of soft tissue or 400 µL of pelleted cultured cells. Keep buffers on ice at all times during the isolation procedure and perform all centrifugation steps at 4 °C in a pre-chilled refrigerated centrifuge. Pre-chill homogenizer and microfuge tubes on ice.

Reagent Preparation

Protease Inhibitor Cocktail Stock Solution (500×)

1. Resuspend the lyophilized Protease Inhibitor Cocktail in 250 µL of anhydrous DMSO.
2. Store at –20 °C.

Homogenization Buffer

1. Prepare sufficient volume of Homogenization Buffer for procedure (~3 mL is required per gram of tissue or milliliter of cell pellet).
2. Add 2 µL of Protease Inhibitor Cocktail Stock Solution to each mL of buffer (1:500).
3. Keep Homogenization Buffer on ice while in use.

Storage Buffer

1. Prepare sufficient amounts of Storage Buffer for procedure (~0.5 mL is required per gram of tissue or milliliter of cell pellet).
2. Add 2 µL of Protease Inhibitor Cocktail Stock Solution to each mL of buffer (1:500).
3. Keep Storage Buffer on ice while in use.

Sample Preparation – Tissue

Notes: For best results, use perfused tissues to eliminate potential contamination by blood. For liver tissue, gently blot the tissue to remove excess moisture and trim away any associated fatty material and connective tissue using surgical scissors. Save a small aliquot of the whole tissue homogenate for further analysis.

1. Place the fresh or thawed frozen tissue (100-400 mg is recommended) in pre-chilled Dounce homogenizer.
2. Add cold Homogenization Buffer (500 µL of buffer per gram of wet tissue) to sample.
3. On ice, gently homogenize tissue sample with 10-15 strokes (the number of strokes for homogenization will vary depending on the tissue type). Add additional Homogenization Buffer (1.5 mL per gram of wet tissue) to the homogenizer, then pipette the tissue slurry up and down several times to fully suspend the homogenate.

Sample Preparation – Cultured Cells

Notes: For cultured cells, it is recommended to use a near-confluent monolayer of cells.
Trypsin/EDTA can be used to detach adherent cells.

1. To prepare cell homogenate, use ~2×10^7 cells.
2. Wash cells once with 1 mL ice cold PBS and centrifuge at 700 × *g* for 5 minutes at 4 °C.
3. Discard the supernatant and resuspend the cell pellet in cold Homogenization Buffer (500 µL of buffer per mL of cell pellet volume).
4. Transfer cell suspension to pre-chilled Dounce homogenizer.
5. On ice, gently homogenize cell suspension with 10-15 strokes (the number of strokes for homogenization will vary depending on the cell line).
6. Add additional Homogenization Buffer (1.5 mL per mL of cell pellet volume) to the homogenizer, then pipette the cell slurry up and down several times to fully suspend the homogenate.

Procedure

Microsome Isolation

1. Transfer the homogenate to a microcentrifuge tube and vortex for 30 seconds, followed by incubation on ice for 1 minute.
2. Centrifuge the homogenate at 10,000 × *g* for 15 minutes at 4 °C.
3. Gently aspirate the thin, floating lipid layer (the "fluffy layer") using a Pasteur pipette, taking care not to aspirate the supernatant. This supernatant is the 'post mitochondrial fraction' (also called the S9 fraction) of the tissue, which contains dilute crude microsomes and cytosolic contents. Save a small aliquot of the S9 fraction for further analysis.
   Note: A small amount of mitochondrial protein may still be detectable in the microsomal fraction. To reduce the likelihood of contamination, it is recommended to completely remove the floating lipid layer from the S9 fraction supernatant. If mitochondrial contamination is a concern, the isolation protocol can be modified to include a second 10,000 × *g* centrifugation step (centrifuge the initial S9 fraction supernatant at 10,000 × *g* for 10 additional minutes at 4 °C and transfer the resultant supernatant to a new microfuge tube).
4. Transfer the supernatant to a new, pre-chilled microcentrifuge tube and centrifuge at maximum speed (≥20,000 × *g*) for 20 minutes at 4 °C.
5. Following centrifugation, aspirate any floating lipids (if needed) and discard the supernatant, taking care to preserve only the light beige/pink opalescent (microsomal) pellet.
   Note: Depending upon the tissue used, a small translucent pellet on the bottom of the microfuge tube may be visible below the microsomal pellet following centrifugation at ≥20,000 × *g*. This is a glycogen pellet. If a glycogen pellet is present, carefully flush the microsomes free from the glycogen with Homogenization Buffer, transfer microsomal suspension to a new microfuge tube and re-centrifuge for 5-10 minutes before proceeding with wash step.
6. Wash the pellet gently with Homogenization Buffer (500 µL per gram of wet tissue or mL of cell pellet volume) and discard the excess buffer. If the microsomal pellet is disturbed during the wash step, re-centrifuge the sample at maximum speed for 5-10 minutes at 4 °C to re-pellet before removing the buffer.

Microsome Storage

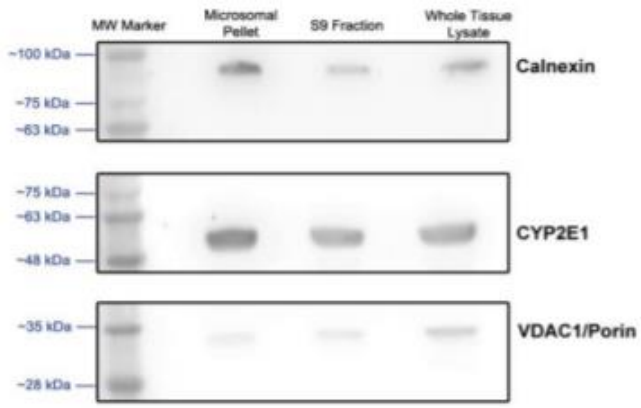
Resuspend the microsomal pellet in ice cold Storage Buffer (500 µL per gram of wet tissue or mL of cell pellet volume) and determine the total microsomal protein concentration. If desired, the protein concentration can be adjusted using additional Storage Buffer. Aliquot the microsomal solution and store at –80 °C for future use.

Notes:

- Activity of microsomal enzymes, including the Cytochrome P450 complex, can be stably maintained for several hours when kept on ice. For long-term enzyme stability, store microsomes at –80 °C. Avoid repeated freeze/thaw cycles.
- For Western blot applications, microsomes can be diluted to an appropriate protein concentration and stored in SDS-PAGE loading buffer.
- For rodent liver tissue, the typical microsomal protein yield is 10–30 mg/mL per gram of liver tissue. This may vary depending upon the individual tissue sample or cell line.

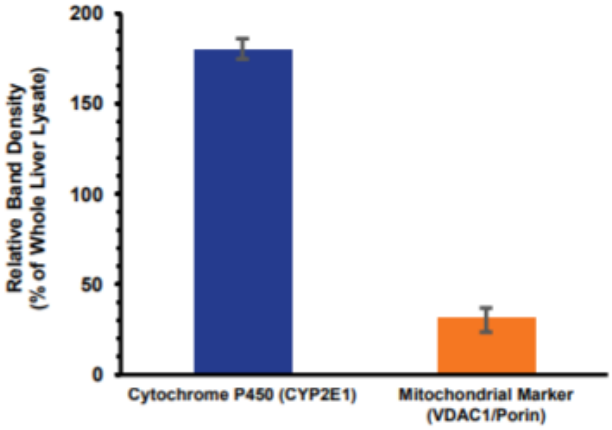
Results

Figure 1.
Western Blot Analysis of Microsomal and S9 Fractions Isolated from Rat Liver.



Microsomes and S9 fraction were isolated according to the procedure for this kit. A total of 30 μg of protein in SDS-PAGE buffer was loaded in each lane and run on a 4–20 % gradient gel. The blots were probed for Cytochrome P450 (CYP2E1), mitochondrial marker VDAC1, and ER-specific protein marker calnexin. Blots show enrichment of CYP and calnexin, and depletion of mitochondrial membrane proteins in microsomal fraction.

Figure 2.
Relative densitometry data



Relative densitometry data demonstrate the enrichment of Cytochrome P450 and reduction of mitochondrial protein marker in microsomal fraction as compared to whole rat liver homogenate (each column shows mean density±SEM relative to whole liver lysate for at least 2 repeats).

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