Microsome Isolation Kit

(Catalog # BN00514; 50 isolations; Store at -20°C)

I. Introduction:

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. Assay Genie's 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.

II. Applications:

- Convenient and fast isolation of microsomal fraction from animal tissues
- Assessment of CYP-mediated drug metabolism and xenobiotic biotransformation
- Protein profiling of microsomal membrane proteins by SDS-PAGE and Western blot

III. Sample Type:

- Mammalian glands and soft tissues such as liver, spleen, lungs etc.
- Cultured eukaryotic cell lines such as HepG2 human hepatic carcinoma cells

IV. Kit Contents:

Components	BN00514	Cap Code	Part Number
Homogenization Buffer	80 ml	NM	BN00514-1
Storage Buffer	20 ml	WM	BN00514-2
Protease Inhibitor Cocktail	1 vial	Red	BN00514-3

V. User Supplied Reagents and Equipment:

- Refrigerated microcentrifuge capable of RCF ≥20,000 x *g*
- Dounce glass tissue homogenizer
- Anhydrous DMSO
- Pasteur pipettes

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Read entire protocol before performing the isolation procedure.

- **Homogenization Buffer and Storage Buffer:** Buffers may be stored at -20°C or 4°C. Keep buffers on ice while in use.
- **Protease Inhibitor Cocktail:** Resuspend the lyophilized Protease Inhibitor Cocktail in 250 µl anhydrous DMSO (not provided) (500X stock solution) and store at -20°C.

VII. Microsome Isolation Protocol:

This protocol is designed for isolation of microsomes from up to 400 mg soft tissue or 400 µl pelleted cultured cells. Before beginning, Prepare sufficient amounts of Homogenization Buffer and Storage Buffer for procedure (~3 ml Homogenization Buffer and 0.5 ml Storage Buffer is required per gram of tissue or milliliter of cell pellet) and add Protease Inhibitor Cocktail to each buffer (1:500; 2 µl per ml for each buffer). 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.

1. **Sample Preparation:** Place the fresh or thawed frozen tissue (~100-400 mg is recommended) in pre-chilled Dounce homogenizer. Add cold Homogenization Buffer (500 µl of buffer per gram of wet tissue) to sample. To prepare cell homogenate, use ~2 x 10^7 cells. Wash cells once with 1 ml ice cold PBS and centrifuge at 700 x *g* for 5 min. at 4°C. Discard the supernatant and resuspend cell pellet in cold Homogenization Buffer (500 µl of buffer per ml of cell pellet volume). Transfer cell suspension to pre-chilled Dounce homogenizer. On ice, gently homogenize tissue sample or cell suspension with 10-15 strokes. Add additional Homogenization Buffer (1.5 ml per gram of wet tissue or per ml of cell pellet volume) to the homogenizer, then pipet the tissue/cell slurry up and down several times to fully suspend the homogenate.

   Notes:

   - For best results, we recommend using perfused tissues, to eliminate potential contamination by blood.
   - Trypsin/EDTA can be used to detach the adherent cells.
   - For cultured cells, we recommend using a near-confluent monolayer of cells.
   - For liver tissue, we recommend gently blotting the tissue to remove excess moisture and trim away any associated fatty material and connective tissue using surgical scissors.
   - The number of strokes for homogenization will vary depending on the tissue type or cell line.

2. **Microsome Isolation:**

   a. Transfer the homogenate to a microcentrifuge tube and vortex for 30 sec., followed by incubation on ice for 1 min. Centrifuge the homogenate at 10,000 x *g* for 15 min. at 4°C.

b. 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.

c. Transfer the supernatant to a new, pre-chilled microcentrifuge tube and centrifuge at maximum speed (≥20,000 x *g*) for 20 min. at 4°C. Following centrifugation, aspirate any floating lipids (if needed) and discard the supernatant, taking care to preserve only the

the light beige/pink opalescent (microsomal) pellet.

d. 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.

Notes:

- Save a small aliquot of the whole tissue homogenate and S9 fraction for further analysis.
- A small amount of mitochondrial protein may still be detectable in the microsomal fraction. To reduce the likelihood of contamination, we recommend completely removing 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 x *g* centrifugation step (centrifuge the initial S9 fraction supernatant at 10,000 x *g* for 10 min. at 4°C and transfer the resultant supernatant to a new microfuge tube).
- If the microsomal pellet is disturbed during the wash step, re-centrifuge the sample at maximum speed for 5-10 min. at 4°C to re-pellet before removing the buffer.
- 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 x *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 min. before proceeding with wash step.

3. 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 hrs. when kept on ice. For long-term enzyme stability, we recommend to store microsomes at -80°C. Avoid repeated freeze/thaw cycles.
- For Western blot applications, microsomes can be diluted to an appropriate protein conc. and stored in SDS-PAGE loading buffer (Cat. no. # 2108 or equivalent).
- 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.

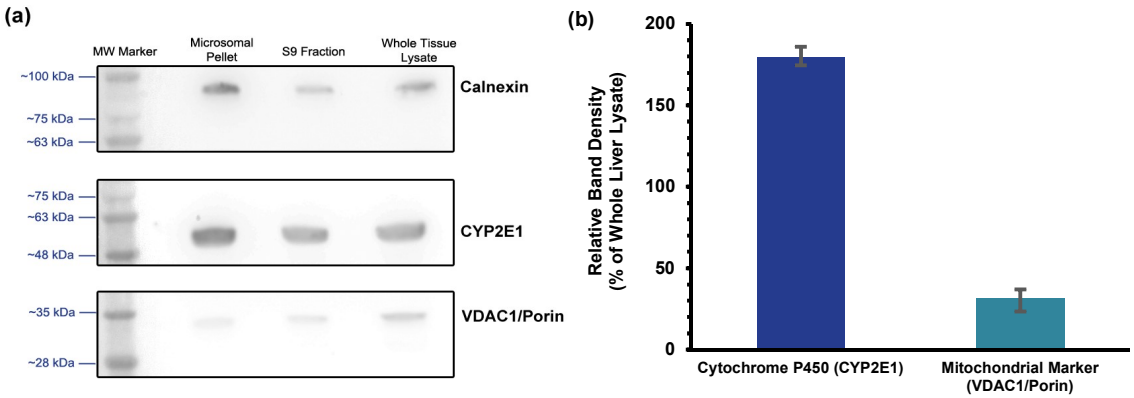

Figure: (a) Western blot analysis of microsomal and S9 fractions isolated from rat liver. Microsomes and S9 fraction were isolated according to the kit protocol described above. A total of 30 µg of protein in SDS-PAGE buffer was loaded in each lane and run on a 4-20% gradient gel. The blots were probed 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. (b) 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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