ab206995 Microsome isolation kit

Instructions for use:

For convenient and fast isolation of microsomal fractions from animal tissues.

View kit datasheet: www.abcam.com/ab206995
(use www.abcam.cn/ab206995 for China, or www.abcam.co.jp/ab206995 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-

Version 5a, Last Updated 05 June 2023

quality products as always, with no changes to specifications or protocols.

Version 5a, Last Updated 05 June 2023

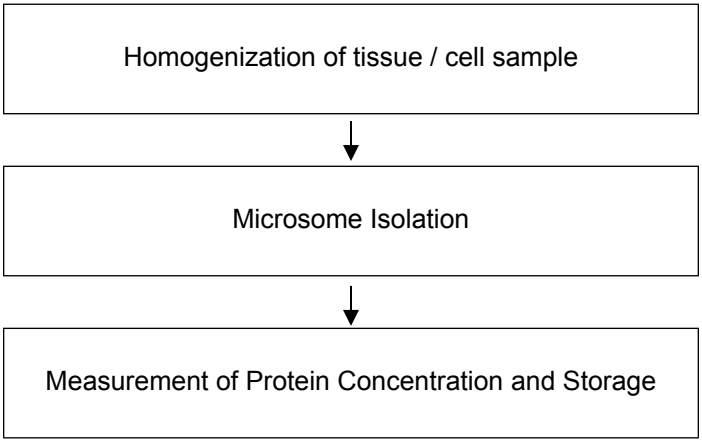
Table of Contents

1. BACKGROUND

Abcam's Microsome Isolation Kit (ab206995) provides a convenient and fast way to isolate microsomal fractions from animal tissues for downstream applications such as assessing CYP-mediated drug metabolism and xenobiotic biotransformation, and protein profiling of microsomal membrane proteins by SDS-PAGE and western blotting.

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* systems 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 b5) required for function of the multicomponent P450 enzyme system. Abcam's Microsome Isolation Kit enables preparation of active microsomes in around 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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 6 and 9.

5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Homogenization Buffer II/Homogenization Buffer	80 mL	-20°C	4°C
Storage Buffer I/Storage Buffer	20 mL	-20°C	4°C
Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail	1 vial	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

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

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single isolation experiment. The starting amount of tissue or cells for a single experiment will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- For best results, use perfused tissues to eliminate potential contamination by blood.
- If using liver tissue, gently blot the tissue to remove excess moisture and trim away any associated fatty material and connective tissue using surgical scissors.

- Trypsin/EDTA can be used to detach adherent cells.

- For cultured cells, use a near-confluent monolayer of cells.
- The number of strokes for homogenization will vary depending on the tissue type or cell line.
- 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, 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 x g centrifugation step (centrifuge the initial S9 fraction supernatant at 10,000 x g for 10 minutes 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 minutes 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 II/Homogenization Buffer, transfer microsomal suspension to a new microfuge tube and re-centrifuge for 5-10 minutes before proceeding with wash step.
- 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.
- Yields may vary depending on the individual tissue sample or cell line. For rodent liver tissue, the typical microsomal protein yield is 10-30 mg/mL per gram of liver tissue.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **Homogenization Buffer II/Homogenization Buffer:**

Ready to use as supplied. Keep on ice while in use.

9.2. **Storage Buffer I/Storage Buffer:**

Ready to use as supplied. Keep on ice while in use.

9.3. **Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail:**

Resuspend the lyophilized Protease Inhibitor Cocktail I/Protease Inhibitor Cocktail in 250 µL anhydrous DMSO (not provided) to produce a 500X stock solution. Keep on ice while in use.

10. SAMPLE PREPARATION

- This protocol is designed for isolation of microsomes from up to 400 mg soft tissue or 400 µL pelleted cultured cells. Before beginning the experiment, prepare sufficient amounts of Homogenization Buffer II/Homogenization Buffer and Storage Buffer I/Storage Buffer for the procedure (approx. 3 mL Homogenization Buffer II/Homogenization Buffer and 0.5 mL Storage Buffer I/Storage Buffer is required per gram of tissue or milliliter of cell pellet). Add Protease Inhibitor Cocktail I/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.

10.1. **Sample types:** Mammalian glands and soft tissues such as liver, spleen and lungs.

10.1.1. Place the fresh or thawed frozen tissue (approx.100 - 400 mg) in pre-chilled Dounce homogenizer.

10.1.2. Add cold Homogenization Buffer II/Homogenization Buffer (500 µL of buffer per gram of wet tissue) to sample.

10.1.3. On ice, gently homogenize tissue sample with 10-15 strokes.

10.1.4. Add more Homogenization Buffer II/Homogenization Buffer (1.5 mL per gram of wet tissue) to the homogenizer. Pipet the tissue/cell slurry up and down several times to fully suspend the homogenate.

10.2. **Sample types:** Cultured eukaryotic cell lines such as HepG2 human hepatic carcinoma cells.

10.2.1. Use approx. 2 x 10^7 cells to prepare cell homogenate.

10.2.2. Wash cells once with 1 mL ice cold PBS.

10.2.3. Centrifuge at 700 x g for 5 minutes at 4°C.

10.2.4. Discard the supernatant. Resuspend cell pellet in cold Homogenization Buffer II/Homogenization Buffer (500 µL of buffer per mL of cell pellet).

10.2.5. Transfer cell suspension to pre-chilled Dounce homogenizer.

10.2.6. On ice, gently homogenize cell suspension with 10-15 strokes.

10.2.7. Add more Homogenization Buffer II/Homogenization Buffer (1.5 mL per mL of cell pellet volume) to the homogenizer. Pipet the cell slurry up and down several times to fully suspend the homogenate.

11. ASSAY PROCEDURE

11.1. Microsome Isolation

11.1.1. Transfer the homogenate to a microcentrifuge tube and vortex for 30 seconds, followed by incubation on ice for 1 minute.

11.1.2. Centrifuge the homogenate at 10,000 x g for 15 minutes at 4°C.

11.1.3. 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 components of the cytosol.

11.1.4. Transfer the supernatant to a new, pre-chilled microcentrifuge tube and centrifuge at maximum speed (≥20,000 x g) for 20 minutes at 4°C.

11.1.5. Following centrifugation, aspirate any floating lipids (if needed) and discard the supernatant, taking care to preserve only the light beige/pink opalescent (microsomal) pellet.

11.1.6. Wash the pellet gently with Homogenization Buffer II/Homogenization Buffer (500 µL per gram of wet tissue or mL of cell pellet volume) and discard the excess buffer.

11.2. Storage

11.2.1. Resuspend the microsomal pellet in ice cold Storage Buffer I/Storage Buffer (500 µL per gram of wet tissue or mL of cell pellet volume).

11.2.2. Determine the total microsomal protein concentration. If desired, the protein concentration can be adjusted using additional Storage Buffer I/Storage Buffer. Aliquot the microsomal solution and store at -80°C for future use.

12. TYPICAL DATA

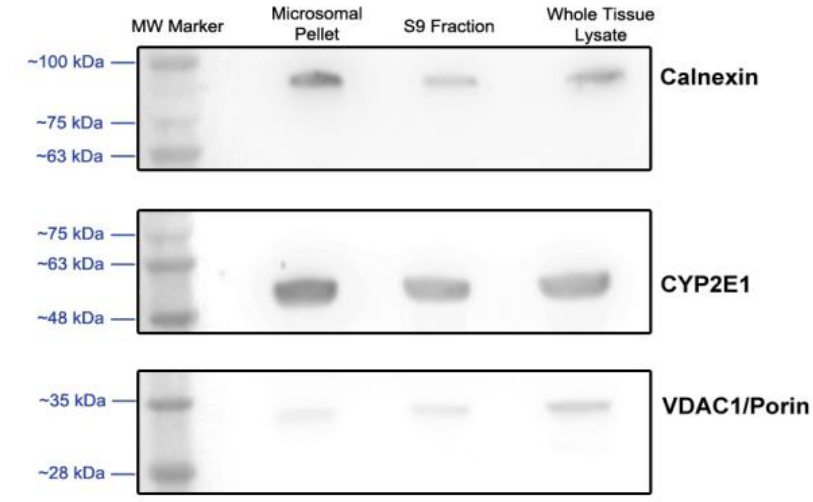


Figure 1: Western blot analysis of microsomal and S9 fractions isolated from rat liver. Microsomes and S9 fraction were isolated according to the kit protocol described above. A total of 30 µg of protein in SDS-PAGE buffer was loaded in each lane and run on a 4-20% gradient gel. The blots were probed for cytochrome P450 (CYP2E1), the mitochondrial marker VDAC1 and the ER-specific protein marker Calnexin. Blots show enrichment of CYP2E1 and calnexin and depletion of mitochondrial membrane proteins in the microsomal fraction.

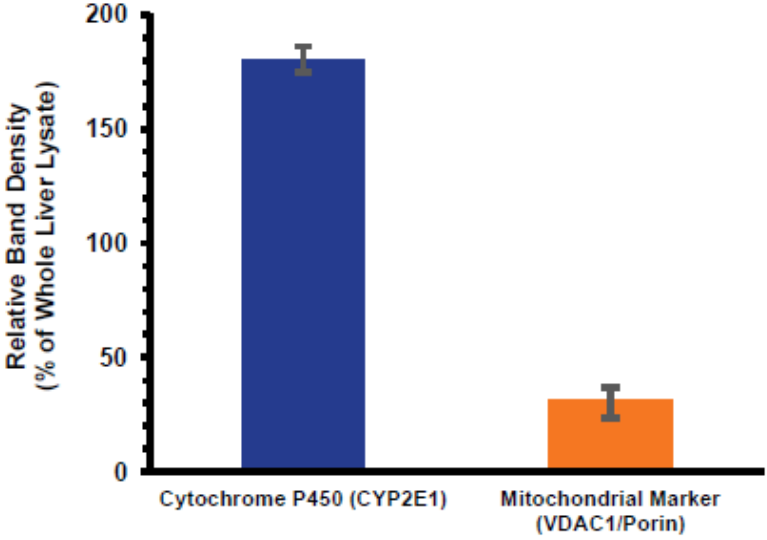


Figure 2: Relative densitometry data demonstrate the enrichment of cytochrome P450 and reduction of mitochondrial protein marker in the microsomal fraction in comparison to whole rat liver homogenate (each column shows mean density ± SEM relative to whole liver lysate for at least 2 repeats).

13. QUICK ASSAY PROCEDURE

NOTE: *This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Homogenize sample with 500 µL of Homogenization Buffer II/Homogenization Buffer per gram of wet tissue or mL of cell pellet using a Dounce homogenizer.
- Add 1.5 mL of Homogenization Buffer II/Homogenization Buffer per gram of wet tissue or mL of cell pellet and resuspend slurry.
- Centrifuge the homogenate at 10,000 x g for 15 minutes at 4°C.
- Gently aspirate off the thin, floating lipid layer, transfer the supernatant to a new microcentrifuge tube and centrifuge at ≥20,000 x g for 20 minutes at 4°C. Aspirate any floating lipids and discard the supernatant.
- Wash the microsomal pellet gently with Homogenization Buffer II/Homogenization Buffer (500 µL per gram of wet tissue or mL of cell pellet volume) and discard the excess buffer.
- Re-suspend the microsomal pellet in ice cold Storage Buffer I/Storage Buffer (500 µL per gram of wet tissue or mL of cell pellet volume).
- Determine the total microsomal protein concentration.

14. NOTES

RESOURCES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)