Microsome isolation kit ab206995

Overview

Product name: Microsome isolation kit
Sample type: Tissue, Suspension cells
Assay time: 0h 45m

Product overview:
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.

The microsome isolation kit enables preparation of active microsomes, 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.

Microsome isolation protocol summary:
- place tissue / washed cells in chilled Dounce homogenizer with homogenization buffer
- homogenize on ice and suspend in buffer
- vortex for 30 s and incubate on ice for 1 min
- spin at 10,000 g for 15 min
- discard floating lipid layer
- spin at 20,000 g for 20 min
- retain pellet
- wash pellet gently with buffer
- resuspend

Notes:
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.

Properties

Storage instructions: Store at -20°C. Please refer to protocols.
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).

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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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<table>
<thead>
<tr>
<th>Components</th>
<th>50 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenization Buffer</td>
<td>1 x 80ml</td>
</tr>
<tr>
<td>Protease Inhibitor Cocktail</td>
<td>1 vial</td>
</tr>
<tr>
<td>Storage Buffer</td>
<td>1 x 20ml</td>
</tr>
</tbody>
</table>
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