

Preparation of Microsomes using FIBERLite F50L-8x39 mL Ultracentrifuge Rotor

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INTRODUCTION

Microsomes are defined operationally as the particulate fraction obtained from a tissue homogenate by ultra centrifugation after the nuclear and mitochondrial fractions have been removed by low speed centrifugation. Electron microscopy has shown that microsomes are composed primarily of closed sacs of membrane called vesicles. Most of the vesicles are derived from rough and smooth endoplasmic reticulum (ER). Membrane vesicles derived from the Golgi apparatus, peroxisomes, endosomes, the trans Golgi network, and other intermediate compartments comprise a minor component of microsomes. Liver microsomes contain rough and smooth ER vesicles in a roughly 2:1 ratio, and, in addition to components of the protein secretory pathway, contain a multitude of proteins involved in lipid/lipoprotein biosynthesis, and drug metabolism. The ER is by far the most abundant membrane in metabolically active cells. Some 2-3 mg of microsomal protein is obtained from liver per gram of wet tissue. As such, microsomes are an ideal preparation in which to study the relationships between enzyme structure, protein-protein and lipid-protein interactions, and the functional properties of membrane bound enzymes. Although many of the most abundant microsomal proteins have been studied extensively, many more remain to be isolated and characterized.

PREPARATION OF MICROSOMES

After a tissue has been selected for study, the composition of the homogenizing buffer, the method of homogenization, and the time and force of the low speed centrifugation step are the primary variables in the preparation of microsomes. The homogenizing buffer is usually isotonic and contains buffer (usually 10-100 mM Tris, HEPES, or Triethanolamine, pH 7.5-8.1), a chelating agent, and a reducing agent such as 1 mM dithiothreitol (DTT). Depending on the tissue selected and the protein or activity of interest, it may be beneficial to add magnesium (1-5 mM), and/or protease inhibitor(s) to the homogenizing buffer. The choice of technique for homogenization is determined by the nature and volume of tissue to be processed. Delicate tissues such as brain and liver are readily homogenized with Potter-Elvehjem tissue grinders. A large volume of tissue is most conveniently homogenized in a Warring blender. Microsomes are pelleted by centrifugation from the post-mitochondrial supernatant at approximately 200,000 g (45,000 RPM) for 30-60 minutes in the Ultracentrifuge using the F50L – 8x39 mL rotor. Rabbit liver microsomes can be prepared by homogenizing minced tissue from two animals (combined liver weight approximately 160 g) in 800 ml 100 mM Tris acetate, pH 7.5, 100 mM KCl, 1 mM EDTA, 0.1 mM DTT in a Warring blender. The livers are perfused with buffer prior to homogenization to limit contamination with hemoglobin and serum proteins. It is important not to over-homogenize the suspension so as to avoid the formation of nuclear and mitochondrial fragments. Differential centrifugation steps are then applied in sequence to



Rotor Model: F50L-8x39 mL



remove unbroken cells, nuclei, and mitochondria. Sequential centrifugation at 600 x g and 10,000 x g gives pellets designated the "nuclear" and "mitochondrial" fractions. Centrifugation of the post mitochondrial supernatant at 105,000 g for ninety minutes yields the microsomes in a pelleted form. The isolated microsomal fraction consists of smooth and rough microsomes, the latter having ribosomes attached on their outer surface. A density gradient sedimentation step can be used at this point to separate the rough and smooth microsomes. Using the same rotor a four – step density gradient can be made in the 39 ml tubes. The w/w density concentrations can be made as follows 20%, 30%, 40% and 50% in 5 ml steps for each concentration and 5 ml of the microsome pellet suspension can be layered on each four-step gradient. After centrifugation at 200,000 x g for 90 min the smooth microsomes can be found at the 30% density range and the rough microsomes can be found 45% density range. Slow acceleration and slow deceleration programs for the ultra centrifuge should be used for the fixed angle rotor to prevent sample/gradient mixing before or after the centrifugation run. A hypodermic syringe and long needle should be used to remove the visible microsome zones in the gradient.

References

1. F.S Heinemann and Juris Ozols, Frontiers in Science 3:483, 1998.

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