**Technical Note:**
**TNCFGLYSENDO 1110**

# Separation of Lysosomes and Endosomes from Rat Liver Homogenate Using Thermo Scientific Fiberlite Carbon Fiber Rotors

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**KEY WORDS**

- Lysosome Separation
- Endosome Separation
- Nycodenz Gradient
- Thermo Scientific WX Ultra Series Centrifuges
- Thermo Scientific Fiberlite F50L-8x39 Rotor


Figure 1: Thermo Scientific Sorvall WX Ultracentrifuge

## Introduction

Cell free systems can be prepared from rat liver to examine the interaction of lysosomes with endosomes[1]. A centrifugation method is used to completely separate lysosomes and endosomes to identify each class of the organelles. The method used is isopycnic separation of the sample after layering on a Nycodenz gradient for centrifugation.

The Thermo Scientific Fiberlite F50L-8x39 rotor and the Thermo Scientific Sorvall WX ultracentrifuge can be used to examine the separated fractions at the maximum g-force of the rotor.

## Materials and Methods

**Preparation of loaded post-mitochondrial supernatant**

Perfuse livers with cold 0.25 M sucrose, 10 mM N-tris (hydroxymethyl) methyl-2-aminoethane-sulphonic acid (TES), pH 7.4, and homogenize in 3 volumes 0.25 M sucrose, 10 m MTES, 1mM MgCl (pH 7.4), and centrifuge at 1,500 xg for 10 minutes at 4 °C.

Incubate the post mitochondrial supernatant at 37°C with 1.3 mM vanadium- free ATP, 10 mM phosphoenol pyruvate, and 35 U of pyruvate kinase to facilitate endosome / lysosomes interaction.

After incubation, chill mixture and centrifuge. Create a four-step discontinuous gradient of equal volumes of 10%, 20%, 30% and 40% Nycodenz layered in polycarbonate (PC) centrifuge tubes as described by Griffith[2]. Layer one milliliter of the endosome/lysosome sample solution on the gradient and centrifuge for 2.5 hours using slow acceleration setting #3 and slow deceleration setting #3 for the ultracentrifuge. These centrifuge settings prevent remixing of the gradients during acceleration and disturbance of the separated zones during deceleration.

## Results

Using a metering peristaltic pump, aspirate the gradient from the centrifuge tube by inserting a long 18 gauge hypodermic needle attached to the pump tubing from the bottom of the centrifuge tube. Collect twenty fractions of equal volumes in small test tubes from each centrifuge tube.

Measure refractive indices of each fraction to identify their exact density of the gradient in each tube. The separation of the endosomal and lysosomal zones can be observed according to their densities as reported by Mullock et al[1].

## Discussion

The Fiberlite® F50L-8x39 carbon fiber ultracentrifuge rotor makes it possible to repeat separations previously done using vertical, near vertical tube and swinging bucket rotors.

The Fiberlite carbon fiber fixed angle rotors can be similarly used for rapid separations of small, precious samples using other gradient media such as sucrose, Ficoll and glycerol, for rate-zonal separation of molecular

forms of estrogen receptors from mammary tumors, nucleic acids (DNA/RNA), viruses and other delicate subcellular organelles as reported previously by Griffith[3].

These carbon fiber rotors also give all the advantages of centrifugation for separation with purification of subcellular particles and the preservation of infectivity of some viruses.

## References


1. Mullock, B. M., Branch, W. J.,van Schaik, M., Gilbert, L.K. and Luzio, J. P., 1989. J. Cell Biol. 108, 2093-2099.
2. Griffith, O.M., Practical Techniques for Centrifugal Separations, 2006, Fiberlite Centrifuge, (Piramoon Technologies Inc): p 39.
3. Griffith, O.M., Density Gradient Separations in Vertical Tube, Near Vertical (NVT), Fiberlite Fixed Angle and Swinging Bucket Rotors: A Comparative Study., 2007, Fiberlite Centrifuge, (Piramoon Technologies Inc), Applications Data A-11342.



Figure 2: Thermo Scientific Fiberlite F50L-8x39 rotor

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