Application Note: AN-LECF-MEMBISOL-0408

Using the Thermo Scientific Sorvall® WX Ultracentrifuge to Isolate Skeletal Muscle Membrane

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Key Words

- Membrane Isolation
- Ultracentrifugation
- Sucrose Gradient
- Fixed-Angle Rotor
- Swinging Bucket Rotor

Introduction

Ultracentrifugation is commonly employed to isolate membrane fractions in sucrose gradients. In order to prepare skeletal muscle membranes for cell biology studies, the Thermo Scientific Sorvall WX ultracentrifuge can be used.

Materials and Methods

Membrane isolation is carried out by a modification of a previous procedure[1-3].

Animals

Fast male Sprague-Dawley rats (250-300 g) for >6 hr, then anesthetize via an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body weight). Excise the gastrocnemius muscles and remove all visible fat, nerve, and vessels. Store the muscles at -80°C until use for membrane preparation.

Plasma membrane isolation:

a. Mince approximately 2 g of rat skeletal muscle in 10 mL of buffer A. Polytron 2 x 1 min at slow speed and bring to a volume of 25 mL. Homogenize further with 10 passes in Potter-Elvehjem tissue grinder. Centrifuge in the WX ultracentrifuge at 34,000 x g for 20 min in the Thermo Scientific T-865 or T-1250 fixed angle rotor.

b. Resuspend precipitate in buffer B and add 2 mL of buffer. Centrifuge at 227,000 x g for 1 hr in the T-865 or T-1250 rotor

c. Resuspend precipitate in buffer B. Add 2000 KU/mL of DNAse and incubate in shaking water bath for 1 hr at 30°C

d. Dilute DNAse mixture 1:2 with ice cold buffer B and cool on ice for 5 min. Layer over top of 16 mL of 34% buffered sucrose. Centrifuge at 135,000 x g for 1 hr in Thermo Scientific SureSpin 630 or AH-629 swinging bucket rotor

e. Remove band and all buffer above it and centrifuge at 227,000 x g for 1 hr in a T-865 or T-1250 rotor

f. Resuspend precipitate in 4 mL of 45% buffered sucrose. Place in centrifuge tube and layer on 2 mL each of 38%, 32%, 30%, 27%, and 1 mL of 12% buffered sucrose. Centrifuge at 68,000 x g for 16 hr in a SureSpin 630 or AH-629 rotor

g. Remove the 27%, sucrose layers and dilute in 20 mM HEPES buffer. Centrifuge at 227,000 x g for 1 hr in a T-865 or T-1250 rotor. Resuspend pellet in 1.0 mL of buffer B

Microsomal membrane isolation:

a. Mince approximately 2 g of rat skeletal muscle in 10 mL of buffer A. Polytron 2 x 1 min at slow speed and bring to a volume of 25 mL. Homogenize further with 10 passes in Potter-Elevehjem tissue grinder. Centrifuge at 34,000 x g for 20 min in a T-865 or T-1250 rotor

b. Centrifuge supernatant at 227,000 x g for 1 hr in a T-865 or T-1250 rotor

c. Resuspend precipitate in buffer D and centrifuge at 227,000 x g for 30 min in a T-865 or T-1250 rotor

d. Resuspend precipitate in buffer E and centrifuge at 227,000 x g for 30 min in a T-865 or T-1250 rotor

e. Resuspend precipitate in 6 mL of distilled water and layer over 10 mL of buffer F. Centrifuge at 135,000 x g for 1 hr in a SureSpin 630 or AH-629 rotor

f. Remove the band and all buffer above it and dilute with distilled water. Centrifuge at 227,000 x g for 1 hr in a T-865 or T-1250 rotor

g. Resuspend the precipitate in 0.3 - 0.4 mL of buffer B to give a final protein concentration of 0.5 - 1.0 mg/mL.

Protein and marker enzyme assay

Use the Bradford method[5] to measure protein concentrations of the membrane fraction, with BSA as the standard. Then determine K^+-stimulated p-nitrophenolphosphatase (KpNPPase) activities as previously described [3].

Buffers

Buffer A: 100 mM Tris HCl, 0.2 mM EDTA, 255 mM Sucrose, pH 7.6
Buffer B: 250 mM Sucrose, 20 mM HEPES, pH 7.4
Buffer C: 3 M KCl, 250 mM Sodium Pyrophosphate
Buffer D: 115 mM Tris HCl, 0.2 mM EDTA, pH 8.2
Buffer E: 255 mM Sucrose, 1 mM Tris HCI, 1 mM $MgCl_2$, pH 8.5
Buffer F: 900 mM Sucrose, 20 mM Tris HCI, 1 mM EDTA, pH 7.4

Results and Discussion

Using the WX ultracentrifuge, skeletal muscle membranes can be isolated and utilized for important biological studies. For instance, isolated membranes have been crucial for examining glucose transport in animals stimulated with insulin or muscle contraction.

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