Minute™ High Fidelity ER Isolation Kit for Liver Tissue

Cat. No. ER-035

Description

Endoplasmic reticulum (ER) is a major membranous structure that functionally connects nuclear membrane and plasma membrane. Liver is an ER-rich organ and has been used for isolation of ER for many decades. Traditional method for isolating ER is based on density gradient ultracentrifugation. The protocol requires large amount of starting material and the methods are tedious and time consuming with significant cross-contamination. Currently, all commercial kits for ER isolation are based on the methods developed in 1970s of last century. Unlike any other ER isolation kit in the market, this kit employees a patented spin-column based technology that is simple, rapid and requires only small amount of tissue. This kit can differentially precipitate native ER (mainly rough ER) from cultured frozen liver tissues without using a Dounce homogenizer and ultracentrifugation. The whole protocol can be done in about 2h.

Kit Components (20 Preps) :

1.	Buffer A	15 ml
2.	Buffer B	4 ml
3.	Buffer C	4 ml
4.	Plastic rods	2
5.	Filter Cartridge	20
6.	Collection Tube	20

Additional Materials Required

1 X PBS, Vortexer, Table-Top Micro centrifuge with maximum speed of 16,000 Xg. **The centrifuge should be able to reach maximum speed within 10 seconds.**

Shipping and Storage: Ship at ambient temperature and store at 4ºC

Important Information:

1. Read the entire procedures carefully. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. All centrifugation steps should be performed at 4ºC in a cold room or in a refrigerated microfuge.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. If protein degradation is a concern, add protease inhibitor cocktails to buffer A prior to use.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Protocol

Note: Warm buffer C to room temperature and mix well prior to use.

1. Place the filter cartridges in a collection tube and incubate on ice.

2. Place 30-35 mg frozen liver tissue (thaw tissue completely at room temperature, fresh tissue is not recommended due to lower final yield and purity) in a filter cartridge. Add 200 µl buffer A to the filter and grind the tissue with a plastic rod for 2-3 min by pushing the tissue against the surface of the filter repeatedly with twisting force After grinding, add 350 µl buffer A to the same filter cartridge (The plastic rod is reusable. Clean it with 70% alcohol or water).

3. Cap the filter cartridge, invert a few times and centrifuge at 16,000 X g for 30 seconds.

4. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds. Centrifuge at 2000 X g for 5 min (the pellet contains nuclei, large cell debris and some un-ruptured cells).

5. Transfer all supernatant to a fresh 1.5 ml microfuge tube (avoid the lipid on the wall of the tube as much as you can) and centrifuged at 4°C for 30 min at 16,000 X g. After centrifugation, carefully transfer 400 µl supernatant to a fresh 1.5 ml tube. The pellet contains mainly larger cell debris, mitochondria, lysosomes and plasma membranes.

6. Add 200 ul buffer B to the tube containing 400 µl supernatant. Mix well by vortexing briefly (buffer B to supernatant ratio is 1:2). Incubate the tube at 4°C for 30 min.

7. Centrifuge at 16,000 X g for 10 min. Remove the supernatant completely. Resuspend the pellet in 200 µl PBS by pipetting up and down for 40-50 times and vortex vigorously for 20 seconds. Incubate at ambient temperature for 15 min, vortex every 5 min. Centrifuge the tube at 2000 X g for 5 min.

8. Transfer the supernatant to a fresh 1.5 ml tube. Add 200 µl buffer C to the supernatant and mix by vortexing briefly (the supernatant to buffer C ratio = 1:1). Incubate the tube at 4°C for 20 min.

9. Centrifuge the tube at 10,000 X g for 10 min. Remove and discard the supernatant. Spin the tube at 10,000 X g for a few second to bring down the residual reagent on the wall of the tube. Remove it completely.

10. Resuspend the pellet (this is isolated ER fraction that contains mainly rough ER) in 50 to 200 µl detergent-containing buffer. If the prep is not used right away, add protease inhibitor cocktails to the prep and store at -80°C. For solubilization of water-insoluble ER fraction reagents in following table are recommended depending upon downstream applications.

Tech Notes

1. The final yield of ER is proportionate to the amount of starting material. If no obvious pellet is seen in step 7 after 16,000 X g spin for 10 min, increase the starting material to 50-80 mg/sample.
2. If more than 50 mg starting material is used and the pellet in step 7 is still very small or not visualized, decrease the centrifugal force from 16,000 X g to 12,000 Xg for 20 min in step 5.
3. Fresh liver sample should be frozen at -20°C or -80°C overnight prior to experiment.
4. If WA-009 is used for solubilization of ER and incomplete solubilization is observed, add SDS to WA-009 to a final concentration of 0.4%.
5. To assess the degree of of ER enrichment, we recommend comparing isolated ER to total tissue lysate in Western blotting (WB) using an ER-specific antibody, such as calreticulin. Ensuring equal protein loading in SDS-PAGE is of utmost importance. We also suggest staining

the post-transfer blot with Ponceau Red to gauge whether there is any notable variation in protein loading.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.