

Minute™ ER Enrichment Kit

(For Tissues and Cultured Cells)

Cat. No. ER-036

Description

Endoplasmic reticulum (ER) is a major membranous structure that functionally connects nuclear membrane and plasma membrane. ER plays a central role in exocytic pathway of protein trafficking in all eukaryotic cells. Proteins synthesized in cytoplasm are translocated into ER from where vesicles shuttle protein cargo to the Golgi apparatus, which subsequently fuse with plasma membrane. Traditional methods for isolating ER are 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, our ER enrichment kit employs a patented spin-column based technology that is simple, rapid and requires only small amount of starting cultured cells or tissues. This kit can differentially precipitate native ER (mainly rough ER) from cultured cells/tissues without using a Dounce homogenizer and ultracentrifugation. The whole protocol can be done in about 2h.

Kit Components (20 prep)

Buffer A	20 ml
Buffer B	1 ml
Buffer C	1 ml
Buffer D	10 ml
Plastic rods	2
Filter Cartridge	20
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 and store at ambient temperature

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 4°C 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

1. Place the filter cartridges in a collection tube and incubate on ice.
 - A. **For cultured cells**, collect 25-35 X 10⁶ cells by low speed centrifugation (500-600 X g for 5 min). Wash cells once with cold PBS. Remove supernatant completely and freeze the pellet at -70-80°C for 10 min. Resuspend the pellet in 550 µl buffer A. **Vortex the tube vigorously for 20-30 seconds**. Immediately transfer the cell suspension to a filter cartridge. Go to step 2.
 - B. **For tissue samples** place 30-40 mg frozen tissue (fresh tissue should be frozen at -20 or -80°C for at least overnight) 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, mix by pipette up and down a few times and go to step 2. **The plastic rod is reusable. Clean it with 70% alcohol or water.**
2. Cap the filter cartridge, invert the tube a few times and centrifuge at 16,000 X g for 30 seconds. (optional: for cultured cells, the flow through in the collection tube can be resuspended and re-pass through the same filter again to increase yield.
3. Without removing the filter cartridge, centrifuge the tube at 2000 X g for 5 min (the pellet contains nuclei, large cell debris and some un-ruptured cells).
4. Discard the filter and transfer all supernatant to a fresh 1.5 ml microfuge tube (try to avoid the lipids as much as possible). Centrifuged at 4°C at 8000 X g for 10 min. After centrifugation, carefully transfer 400 µl supernatant to a fresh 1.5 ml tube (try to avoid the lipids). The pellet contains mainly larger cell debris, mitochondria, lysosomes and plasma membranes.
5. Add 40 ul buffer B to the 400 µl supernatant. Mix well by vortexing briefly (buffer B to supernatant ratio is 1:10). Incubate the tube at 4°C for 20-30 min.
6. Centrifuge at 8000 X g for 10 min. Remove the supernatant completely. Resuspend the pellet 400 µl cold buffer A by pipetting up and down for 40-50 times and vortex vigorously for 20 seconds. Add 40 ul of buffer C (1/10 of resuspended volume) to the tube and vortex briefly. Incubate at room temperature for 10-15 min. Vortex the tube every five min. Centrifuge the tube at 8000 X g

for 5 min. Transfer 400 µl supernatant to a fresh 1.5 ml tube. Add 400 µl buffer D to the supernatant and mix by vortexing briefly (the supernatant to buffer D = 1:1). Incubate the tube at 4°C for 20 min.

7. 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.
8. Resuspend the pellet in 50 to 200 µl PBS (this is isolated ER fraction that contains mainly rough ER. If the prep is not used right away, add protease inhibitor cocktails to the prep and store at -80°C). The ER content varies significantly in different type of cells/tissues. The isolated ER yield is typically 20-200 µg/sample. The water insoluble ER fraction can be dissolved in any buffer of your choice but following reagents are recommended depending upon downstream applications.

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.