# Minute™ ER Enrichment Kit
## for Tissues and Cultured Cells

**Cat. No. ER-036**

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### Description

The endoplasmic reticulum (ER) is a major membranous structure that functionally connects the nuclear membrane and plasma membrane. ER plays a central role in the exocytic pathway of protein trafficking in all eukaryotic cells. Proteins synthesized in the cytoplasm are translocated into ER from where vesicles shuttle protein cargo to the Golgi apparatus, which subsequently fuse with the plasma membrane. Traditional methods for isolating ER are based on density gradient ultracentrifugation. The protocol requires a 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 the 1970s of the last century. Unlike any other ER isolation kit in the market, Minute™ ER enrichment kit employs a patented spin-column-based technology that is simple, rapid, and requires only a 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 (20T):

| | | |
|---|---|---|
| 1. | Buffer A | 20 ml |
| 2. | Buffer B | 1 ml |
| 3. | Buffer C | 1 ml |
| 4. | Buffer D | 10 ml |
| 5. | Plastic rods | 2 |
| 6. | Filter Cartridge | 20 |
| 7. | 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 4°C.

### 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**.

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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 D to room temperature and mix well prior to use.**

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

A. **For cultured cells**:collect 25-35 X $10^6$ 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, and invert the tube a few times followed by centrifugation at 16,000 X g for 30 seconds.
   ***(Optional: the flow through in the collection tube can be resuspended and re-pass through the same filter again to increase yield.)***

3. Discard the filter and resuspend the pellet by vigorous vortexing for 10 seconds. Centrifuge at 2,000 X g for 3 min (the pellet contains nuclei, large cell debris and some un-ruptured cells).

4. Transfer all supernatant to a fresh 1.5 ml microfuge tube (for liver tissue, try to avoid the lipids as much as possible) and centrifuge at 4°C at 8,000 X g for 10 min. After centrifugation, carefully transfer 400 µl supernatant to a fresh 1.5 ml tube (try to avoid the lipids if they are present). The pellet contains mainly larger cell debris, mitochondria, lysosomes, and plasma membranes.

5. Add 40 µl 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 8,000 X g for 10 min. Remove the supernatant completely, and resuspend the pellet in 400 µl cold buffer A by pipetting up and down for 40-50 times followed by vigorous votexing for 20 seconds. Add 40 µl of buffer C (1/10 of resuspended volume) to the tube and vortex briefly. Incubate at room temperature for 10-15 minutes (**vortex the tube every five min**). Centrifuge at 8,000 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 at 10,000 X g for 10 min. Remove and discard the supernatant. Spin the tube at 10,000 X g briefly to bring down the residual aqueous to be removed completely.

8. Resuspend the pellet in 50 to 200 µl PBS (this is an 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 types of cells/tissues. The isolated ER yield is typically 20-200 µg/sample. The water-insoluble ER fraction can be solubilized in any buffer of your choice, however, the below-listed reagents are recommended depending upon downstream applications.

**Tech Note:**

1. In most cases, the crude ER pellet is visible after 8,000 X g centrifugation in step 6. However, the ER pellet may be more transparent and may not be seen easily for some cell types. If this is the case, the incubation time in step 5 can be increased to 1h, and the centrifugation force in step 6 can be increased to 10,000 X g. Even if the pellet is not very obvious, you can assume that ER pellet is there and continue the protocol to the end. Be sure to wash the wall of the tube in step 6 with 400 µl buffer A.
2. If cultured cells are used and the final ER yield is less than 20 µg, increase the starting cell# to 50 million. Pellet the cells with low speed centrifugation as described in step 1A. Reuspend the cell pellet in 100 µl buffer A and freeze it at -20°C for 1h. Thaw out cell suspension, pipette up and down for 30-40 time and transfer cell suspension to the filter. Homogenize the cell suspension in the filter cartridge by pushing the plastic rod against the surface of the filter with twisting force for about 100 times. Add 400 µl buffer A to the filter cartridge and proceed to step 2.

**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. |