ReadiPrep™ Endoplasmic Reticulum (ER) Isolation Kit

Catalog number: 67302
Unit size: 100 tests

Component	Storage	Amount (Cat No. 67302)
Component A: Isotonic Extraction Buffer (5X)	Refrigerated (2-8 °C)	One Bottle (20 mL)
Component B: Hypotonic Extraction Buffer (10X)	Refrigerated (2-8 °C)	One Bottle (10 mL)
Component C: 800 mM CaCl2 Solution	Refrigerated (2-8 °C)	One Bottle (10 mL)

OVERVIEW

ReadiPrep™ Endoplasmic Reticulum (ER) Isolation Kit is a complete reagent system designed for the enrichment and purification of endoplasmic reticulum from mammalian tissues and cultured cells. The ER is essential for various cellular processes including protein synthesis, calcium storage, lipid metabolism, and membrane protein trafficking. This kit enables rapid isolation of intact ER structures including rough and smooth ER without the need for ultracentrifugation.

The protocol yields multiple levels of ER enrichment, from crude microsomes to calcium-precipitated and density-gradient purified fractions. The resulting ER fractions are functionally intact and well suited for studies involving protein synthesis, degradation pathways, ER-resident enzymes, and organelle-specific biochemical assays. ReadiPrep™ ER Isolation Kit simplifies ER purification into a streamlined workflow, making it ideal for routine laboratory use.

AT A GLANCE

1. Detach cells and centrifuge the cells at 600 X g for 5 minutes, wash with PBS and measure the packed cell volume (PCV).
2. Suspend the cells in 1X Hypotonic Extraction Buffer equivalent to 3X PCV measured, incubate for 20 minutes at 4 °C.
3. Centrifuge the cells at 600 X g for 5 minutes, and remove the supernatant by aspiration, measure the packed cell volume (PCV).
4. Add 1X Isotonic Extraction Buffer equivalent to 2X the PCV measured, break the cells by sonication.
5. Centrifuge the homogenate at 1,000 X g for 10 minutes at 4°C. Collect the supernatant.
6. Centrifuge at 12,000 X g for 15 minutes at 4°C. Collect the supernatant, which is the post-mitochondrial fraction (PMF).
7. Add Calcium Chloride Solution at final concentration of 7 mM CaCl2. Incubate for 10 minutes at 4°C.
8. Item content
9. Centrifuge the sample at 8,000 X g for 10 minutes at 4°C.
10. Suspend the pellet in 200 to 300 µL of 1X Isotonic Extraction Buffer. Store at -80°C.

PREPARATION OF WORKING SOLUTION

1X Isotonic Extraction Buffer

Dilute 20 mL 5X Isotonic Extraction Buffer (Component A) in 80 mL water to make 1X Isotonic Extraction Buffer (total volume 100 mL). Keep the 1X Isotonic Extraction Buffer at 4°C. Add 1mL Protease Inhibitor Cocktail (Sigma, Cat#P8340-1ML, not provided, or other preferred protease inhibitors) to the 1X Isotonic Extraction Buffer at a concentration of 1% (v/v) before use. Prepare the volume as needed.

1X Hypotonic Extraction Buffer

Dilute 10 mL 10X Hypotonic Extraction Buffer (Component B) in 90 mL water to make 1X Hypotonic Extraction Buffer (total volume 100 mL). Keep the 1X Hypotonic Extraction Buffer at 4°C., Add 1 mL Protease Inhibitor Cocktail (Sigma, Cat#P8340-1ML, not provided, or other preferred protease inhibitors) to the 1X Isotonic Extraction Buffer at a concentration of 1% (v/v) before use. Prepare the volume as needed.

8 mM CaCl2 Solution

Dilute 1 mL CaCl2 Solution (Component C) in 99 mL water to prepare 8.0 mM CaCl2 solution. 7.5 mL of the 8 mM Calcium Chloride Solution is enough for each mL of post-mitochondrial fraction (PMF). Prepare the volume as needed.

SAMPLE EXPERIMENTAL PROTOCOL

Note: This protocol can be used for adherent cells (such as HeLa cells).

Step I: Prepare crude ER (microsomes):

1. Detach the cells 10 cm plates) using conventional tissue culture methods.
2. Centrifuge the cells at 600 X g for 5 minutes. Remove the supernatant by aspiration.3. Wash the cells with 10 volumes of PBS.
3. Measure the packed cell volume (PCV).
4. Suspend the cells in a volume of 1X Hypotonic Extraction Buffer equivalent to 3X PCV measured.
5. Incubate the cells for 20 minutes at 4°C to allow the cells to swell.
6. Centrifuge the cells at 600 X g for 5 minutes and remove the supernatant by aspiration.
7. Measure the "new" PCV.
8. Add a volume of 1X Isotonic Extraction Buffer equivalent to 2X the "new" PCV.
9. Sonicate on ice for three cycles to break the cells.
10. Centrifuge the homogenate at 1,000 X g for 10 minutes at 4°C. Carefully remove the thin floating lipid layer by aspiration, being careful not to aspirate the post-nuclear supernatant. Transfer the supernatant to another centrifuge tube using a pipette, and discard the pellet.
11. Centrifuge at 12,000 X g for 15 minutes at 4°C. Carefully remove the thin floating lipid layer by aspiration, being careful not to aspirate the post mitochondrial supernatant. Transfer the supernatant (650 µL) to another tube using a pipette. Discard the pellet. This supernatant fraction, which is the post-mitochondrial fraction (PMF), is the source for microsomes.

Step II: Isolation of Ca2+-precipitated Rough ER (RER) enriched microsomes:

1. Measure the volume of the PMF [Step I (12), 650 µL].
2. Prepare a volume of 8 mM Calcium Chloride Solution equivalent to 7.5X the volume of the PMF (4.875 mL).
3. Add the above volume of 8 mM Calcium Chloride Solution to the PMF dropwise with constant stirring. The final concentration of CaCl2 is 7 mM.

© 2025 AAT Bioquest, Inc. Last revised August 2025. For more information and tools, please visit https://www.aatbio.com

4. After all of the 8 mM Calcium Chloride Solution is mixed with the PMF, Incubate for 10 minutes at 4 °C.
5. Centrifuge the sample at 8,000 X g for 10 minutes at 4°C. The enriched RER microsomes will be in the pellet.
6. Remove the supernatant. Suspend the pellet in 200 µL of 1X Isotonic Extraction Buffer.
7. Store at -80 °C.

EXAMPLE DATA ANALYSIS AND FIGURES

I: Cytochrome c Reductase Assay in purified microsomes (endoplasmic reticulum, ER):

Monitoring ER isolation process by measuring NADPH-Cytochrome c reductase activity:

Reagents or Equipment Needed:

- Assay Buffer: 300 mM Potassium Phosphate buffer, pH 7.8, containing 0.1 mM EDTA and 0.5 mg/ml BSA
- Cytochrome c: 45 mg/mL in H2O (MW = 12 kDa, 45 mg/ml = 3.75mM)
- NADPH: 10 mM in H2O
- 96-well plate (Clear bottom)
- Absorbance microplate reader

Prepare Sample:

Dilute ER sample 2-10X in Assay Buffer, 100µL /test

Prepare Working Solution:

0.9 mg/ml Cytochrome c in Assay Buffer

Cytochrome c Reductase Assay Protocol:

1. Set the spectrophotometer to 550 nm.
2. Place 100 µL of sample in a clear 96 well plate, for a blank control, add 100 µL assay buffer without ER sample present.
3. Add 80 µLof Working Solution to each well.
4. Set up kinetic program for 30 minutes with one minute interval.
5. Add 20 µL of10 mM NADPH solution to each well.
6. Measure the absorbance at 550 nm.
7. Calculate the activity of the samples.

Calculation:

Unit definition: One unit will reduce 1.0 µmole of cytochrome c in the presence of 1 mM NADPH per minute at pH 7.8 at 25 °C.

$$\text{Units/ml} = \frac{(\Delta A_{550}/\text{min} \times DF \times 0.2)}{(\text{Sample Vol} \times 21.1)}$$

ΔA550/min = (ΔA(sample) – ΔA (blank))/min
(It is the slope of the linear range in Absorbance-Time progress curve)

DF = dilution factor of sample

0.2 = reaction volume in ml

Sample vol = volume of sample or enzyme in ml

21.1 = Extinction coefficient (ℰmM) for reduced cytochrome c

Example of activity of the samples assayed:

$$\text{Units/ml} = \frac{(0.0059 \times 2 \times 0.2)}{(0.1 \times 21.1)}$$

$$= 1.118 \times m \text{ Units/ml}$$

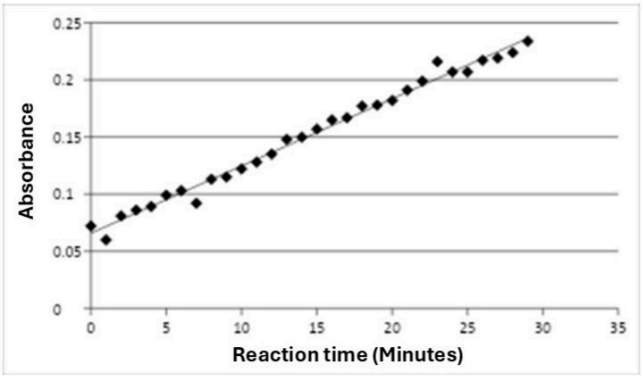
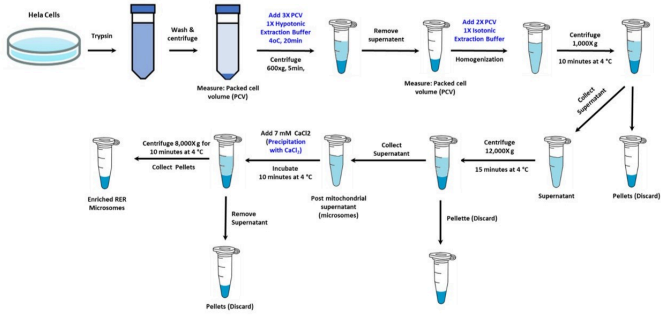


Figure 1. Progress curve of ER Cytochrome C reductase activity measurement. The velocity is obtained from the slope of the linear part of the curve, referred to a distinct time unit (min). The velocity is derived from the slope of a line connecting this point with the blank at the start of the reaction. The NADPH-Cytochrome c reductase activity (1.118 x mU /ml) was determined from the isolated ER sample using ER isolation kit (Cat# 67302).

APPENDIX

Preparations of Endoplasmic Reticulum (ER)



DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

© 2025 AAT Bioquest, Inc. Last revised August 2025. For more information and tools, please visit https://www.aatbio.com