PRODUCT INFORMATION & MANUAL

Endoplasmic Reticulum Enrichment Kit

NBP2-29482

Research use only. Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt.

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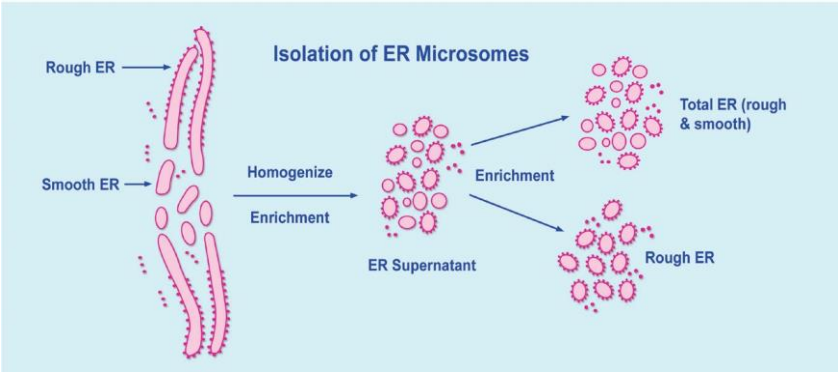
I. OVERVIEW

The Endoplasmic Reticulum (ER) is a membranous labyrinth that extends throughout the cytoplasm of the cell as a single continuous network of flattened sacs and tubules. The ER accounts for more than half the total membrane in eukaryotic cells and 10% of a cell's total volume, thus representing one of the largest organelles. It plays a critical role not only in lipid and protein biosynthesis but is central to protein modification, folding, and assembly. The ER is generally characterized as being devoid of membrane-bound ribosomes, (smooth ER), or studded with ribosomes (rough ER); each differing in structure, appearance, and function.

Although isolating the ER may seem difficult due to its size and complex association with other components of the cytosol, the Endoplasmic Reticulum Enrichment Kit is a key tool that provides the necessary reagents together with an optimized protocol required to gently isolate and enrich for ER from tissues.

This easy-to-use, gentle enrichment method is ideal where isolated ER microsomal fractions are desired, but density gradient purified preparations are not required. The isolated ER microsomal fractions may be used in understanding biochemical processes associated with the ER such as protein synthesis, lipid synthesis, unfolded protein response, cellular stress, and more.

This kit is routinely tested and standardized using 0.5 gram of mouse liver tissue. Representative data is shown in Figs. 1 and 2. Researchers should adjust and/or optimize their methods according to starting materials.



II. QUALITY CONTROL

The kit is routinely tested using 0.5 gram of freshly isolated mouse liver tissue as the starting material. We typically get the following yields:

Total ER fraction: 350 µL (12 mg/mL = 4.2 mg) or
Rough ER fraction: 300 µL (9 mg/mL = 2.7 mg)

These yields are based on using the 0.5 gram starting material for either isolating the Total ER fraction or the Rough ER fraction. If the starting material is divided at Step C and both the Total and Rough ER fractions are isolated, then yields will be approximately ½. Please note that all yields are approximate and may vary depending on the tissue type used and other variables.

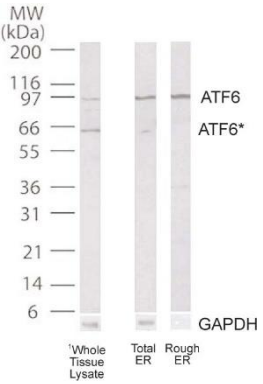


Figure 1: Western blot analysis of ATF6 (NBP1-40256, 3 µg/mL) and GAPDH (NB100-56875, 0.25 µg/mL). ATF6 is an ER stress-regulated transmembrane transcription factor. The blots were probed with ATF6, stripped and re-probed with GAPDH. Full-length ATF6 was detected in all 3 lanes and was enriched in the ER fractions. GAPDH was detected in the whole lysate and total ER fraction, but not in the rough ER fraction. ATF6* may represent partial or cleaved/active ATF6.

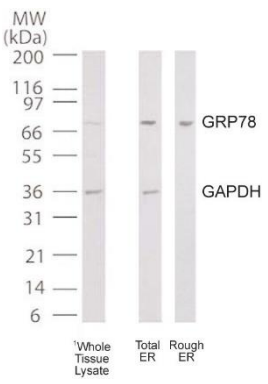


Figure 2: Western blot analysis of GRP78 (NB100-56411, 1 µg/mL) and GAPDH (NB100-56875, 0.25 µg/mL). GRP78 is an ER chaperone protein. The blots were simultaneously probed with GRP78. GRP78 was detected in all 3 lanes and was enriched in the ER fractions. GAPDH was detected in the whole lysate and total ER fraction, but not in the rough ER fraction.

[1]Whole mouse liver lysate was prepared using the Tissue Lysate Preparation Protocol
Total and Rough mouse liver ER fractions were prepared using the Endoplasmic Reticulum Enrichment Kit (Catalog no. NBP2-29482). 20 µg protein was loaded on each lane of a 10 well SDS-mini PAGE gel. Each set of blots was probed with an anti-ER protein and GAPDH. GAPDH is a cytoplasmic protein often used as a protein loading control.

III. KIT COMPONENTS AND STORAGE

Components and storage for the Endoplasmic Reticulum Enrichment Kit

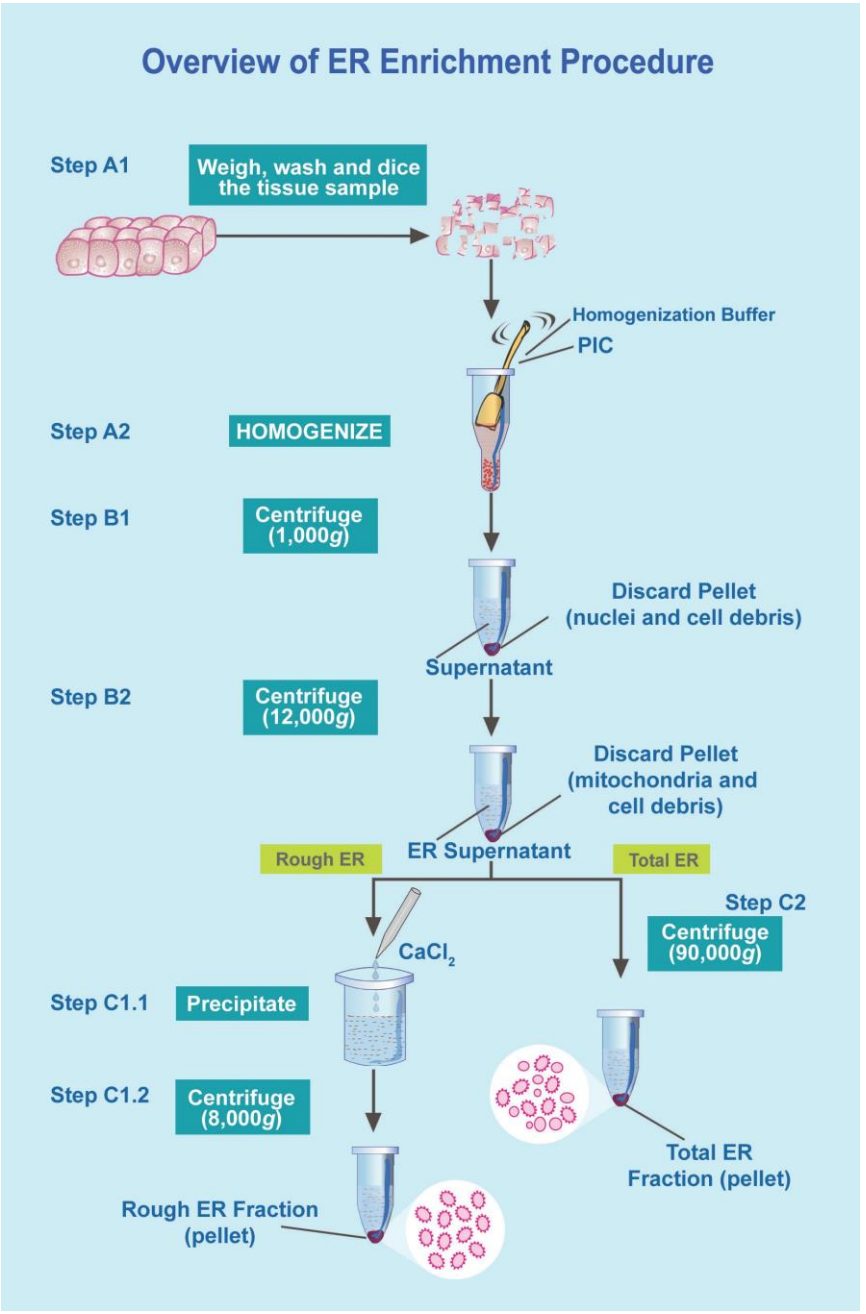
REAGENTS (4 °C STORAGE)		
KC-414	5X Isosmotic Homogenization Buffer (HEPES, Sucrose, KCl)	30 mL
KC-415	1X Suspension Buffer (HEPES, Sucrose, KCl, EGTA)	30 mL
KC-117	10X PBS 2 x 50 mL	
KC-416	5X Calcium Chloride Solution ($CaCl_2$)	60 mL
REAGENTS (-20 °C STORAGE NON-FROST-FREE)		
KC-121	100X Protease Inhibitor Cocktail (PIC) 2 x 0.75 mL	

Additional Reagents/Equipment required but not provided:

- Sterile water
- Standard balance
- High-speed centrifuge (Beckman GS-15R centrifuge, Beckman Avanti J30I centrifuge with JS24 rotor or equivalent)
- Compatible centrifuge tubes
- Glass homogenizer (15-mL Wheaton tube and Pestle or equivalent for 1 gram of tissue sample)
- Microcentrifuge tubes

IV. REAGENT PREPARATION

1X Isosmotic Homogenization Buffer	Add 4 mL of sterile water per 1 mL of 5X Isosmotic Homogenization Buffer (KC-414)
1X PBS	Add 9 mL of sterile water per 1 mL of 10X PBS (KC-117)
1X $CaCl_2$	Add 4 mL of sterile water per 1 mL of 5X $CaCl_2$ (KC-416)


Overview of ER Enrichment Procedure
Step A1
Weigh, wash and dice the tissue sample
Homogenization Buffer
PIC
Step A2
HOMOGENIZE
Step B1
Centrifuge (1,000g)
Discard Pellet (nuclei and cell debris)
Supernatant
Step B2
Centrifuge (12,000g)
Discard Pellet (mitochondria and cell debris)
Rough ER
ER Supernatant
Total ER
Step C2
Centrifuge (90,000g)
CaCl2
Step C1.1
Precipitate
Step C1.2
Centrifuge (8,000g)
Total ER Fraction (pellet)
Rough ER Fraction (pellet)

V. PROCEDURE FOR ER ENRICHMENT FROM ANIMAL TISSUES

Please note: All the steps should be performed at 4 ° C using pre-chilled solutions and materials. If Starting Material is less than or more than 0.5 gram, adjust reagent volumes accordingly.

A. Tissue Homogenization/Cytoplasmic Collection

1. Weigh tissue (this will be referred as Starting Material; this protocol is optimized for 0.5 gram), wash in 10 mL of 1X PBS and cut into small pieces (0.3-0.5 cm) using a clean razor blade. Collect cut pieces in a clean homogenizer.
2. Add 2 mL of 1X Isosmotic Homogenization Buffer followed by 20 µL of 100X PIC per 0.5 gram of tissue. Homogenize using 20 to 30 strokes.
3. Transfer the homogenate to a centrifuge tube. Wash the pestle with 0.5 mL of 1X Isosmotic Homogenization Buffer and add the residual homogenate to the homogenate in the centrifuge tube.

B. Pelleting out Nuclear, Mitochondrial, and Cell Debris

1. Centrifuge the homogenate at 1,000g (Beckman GS-15R Centrifuge or equivalent) for 10 min at 4 ° C. Carefully remove the thin floating lipid layer (if present) and discard. Transfer the supernatant to a clean centrifuge tube for Step B2. Discard the pellet (nuclei and cell debris).
2. Centrifuge the supernatant at 12,000g (Beckman Avanti J30I centrifuge with JS24 rotor or equivalent) for 15 min at 4 ° C. Carefully remove the thin floating lipid layer (if present) and discard. Transfer the supernatant to a centrifuge tube for Step C. Discard the pellet (mitochondria and cell debris).

C. Enrichment of Total ER and Rough ER

Rough ER Fraction
Use the supernatant obtained from Step B2; Refer to Table 1 for reagent volumes and proceed to Step C1.

Total ER Fraction
Use the supernatant obtained from Step B2; Refer to Table 2 for reagent volumes and proceed to Step C2.

C1. Rough ER Fraction

1. Transfer the ER supernatant (Step B2) to a beaker suitable for use on a magnetic stirring plate. Put a spin bar in the beaker, put the beaker in ice and add 1X $CaCl_2$ solution drop by drop for precipitation while the supernatant stirs for 15 min.
2. Transfer the supernatant from the beaker to a centrifuge tube. Centrifuge at 8,000g (Beckman Avanti J30I centrifuge with JS24 rotor or equivalent) for 10 min at 4 °C.
3. Remove the supernatant and suspend the pellet (which contains Rough ER Fraction) in 1X Suspension Buffer and add 100X PIC. This step might need mild to vigorous vortexing if the pellet does not suspend in the buffer easily. Store at -80 °C, avoid repeated freeze/thaw.

Table 1.

Step	Reagent	Rough ER Fraction
C1.1	1X $CaCl_2$	15 times the volume of the supernatant from Step B2
C1.3	1X Suspension Buffer	0.30 mL/0.5 gram of Starting Material (Step A1)
C1.3	100X PIC	5 µL/0.5 gram of Starting Material (Step A1)

C2. Total ER Fraction

Transfer the ER supernatant (from Step B2) to a microcentrifuge tube, centrifuge at 90,000g (Beckman Avanti J30I centrifuge with JS24 rotor or equivalent) for 60 min at 4 °C. Remove the supernatant and discard. Suspend the pellet (which contains the Total ER Fraction) in 1X Suspension Buffer and add 100X PIC. This step might need mild to vigorous vortexing if the pellet does not suspend in the buffer easily. Store at -80 °C, avoid repeated freeze/thaw.

Note: If both Rough ER & Total ER fractions are desired, split the supernatant obtained from Step B2 into 2 equal fractions and process each fraction accordingly. It will be necessary to adjust reagent volumes accordingly.

Table 2.

Step	Reagent	Rough ER Fraction
C2.1	1X Suspension Buffer	0.30 mL/0.5 gram of Starting Material (Step A1)
C2.3	100X PIC	5 µL/0.5 gram of Starting Material (Step A1)

VI. RELATED ANTIBODIES

Description	Cat. No.	Format	Clone	Qty	Species	Application
ATF6 (Full-length and Active/Cleaved Forms)	NBP1-40256	Purified	70B1413.1	0.1 mg	Multi	ChIP, IF/ICC, IHC-P, IP, WB
BiP/GRP78	NB100-56411	Purified	N/A	0.1 mg	Human	WB
GAPDH - Loading Control	NB300-320	Purified	N/A	0.1 mg	Multi	IHC-P, WB
GAPDH - Loading Control	NB300-221	TC SUP	GAPDH 1D4	0.1 mg	Multi	IF/ICC, WB

VII. GENERAL REFERENCES

The protocol was optimized by gathering collective information from the following references:

1. Plonne, D., Cartwright, I. J., Linb, W., Dargel, R., Graham, J. M., Higgins, J. A. 1999. Separation of the intracellular secretory compartment of rat liver and isolated rat hepatocytes in a single step using self-generating gradients of iodixanol. Anal. Biochem. 276:88-96.
2. Szarka A, Stadler K, Jenei V, Margittai E´, Csala M, Jakus J, Mandl J, Ba´nhegyi G. 2002. Ascorbyl free radical and dehydroascorbate formation in rat liver endoplasmic reticulum. J Bioenerg Biomembr. 34:317–23.
3. Current Protocols in Cell Biology 37:3.27.1-3.27.23. ©2007 by John Wiley & Sons, Inc.

VIII. PRODUCT CITATION

1. Pancreas-specific aquaporin 12 null mice showed increased susceptibility to caerulein-induced acute pancreatitis. Ohta E, T Itoh, T Nemoto, J Kumagai, SBH Ko, K Ishibashi, M Ohno, K Uchida, A Ohta, E Sohara, S Uchida, S Sasaki, T Rai. Am J Physiol. doi:10.1152/ajpcell.00117.2009 (2009).

Novus product cited: Endoplasmic Reticulum (ER) Enrichment Kit (NBP2-29482): WB (rat pancreas), Fig. 9. The results show that APQ12 was enriched in the rough ER.

2. Ins(1,4,5)P3 facilitates ATP accumulation via phosphocreatine/creatine kinase in the endoplasmic reticulum extracted from MDCK cells. Sun J, S Ogata, M Segawa, S Usune, Y Zhao, T Katsuragi. Biochem Biophys Res Communications 397:465-465 (2010).

The ER Kit, Catalog no. NBP2-29482 was used to isolated ER from MDCK (NBL-2) canine kidney cells:

Fig. 1A, Electron microscopy of isolated ER samples. The typical structural features of ER are seen.

Fig. 1B, WB for protein markers of the ER membrane using calnexin (90 kDa), Derlin1 (22 kDa) and VIMP (21 kDa) antibodies. These proteins were detected in the microsome, but not cytosol fractions.

Figs 2-4. Determination of ATP content in fresh frozen ER samples. The results showed that the ER fresh and frozen isolates contained ATP. ATP accumulation in the ER could be manipulated by cell treatments prior to ER isolation.

3. Comprehensive analysis of endoplasmic reticulum-enriched fraction in root tips of soybean under flooding stress using proteomics techniques. Komatsu S, R Kuji, Y Nanjo, S Hiraga, K Furukawa. J Proteomics http://dx.doi.org/10.1016/j. jprot.2012.09.032 (2012).

The ER Kit, Catalog number NBP2-29482 was used to isolated ER from the root tips of soybeans.

Isolation scheme diagram (S1), WB (Figs 1, S2), Gel and 1D gel-free based proteomics techniques using mass spectrometry (Figs 2,3; Tables 1-4).

The results suggested that flooding stress suppressed protein synthesis and glycosylation in the ER in the root tips of soybeans.

4. Mesenchymal stem cells and endothelial progenitor cells decrease renal injury in experimental swine renal artery stenosis through different mechanisms.
Zhu X-Y, V Urbieta-Caceres, JD Krier, SC Textor, A Lerman, LO Lerman. Stem Cells 31:117-125 (2013).

Novus product cited: Endoplasmic Reticulum (ER) Enrichment Kit (NBP2-29482) for WB (pig kidney): Fig 4D, ER fractions were analyzed by WB with various antibodies.

5. Reconstructed glycan profile for evaluation of operating status of the endoplasmic reticulum glycoprotein quality control. Iwamoto S, M Isoyama, M Hirano, K Yamaya, Y Ito, I Matsuo, K Totani. Glycobiology 23:121-131 (2013).

Novus product cited: Endoplasmic Reticulum (ER) Enrichment Kit (NBP2-29482) for mouse and rat liver:

1. WB (Fig. S1): ER fractions were analyzed with various ER and Golgi marker antibodies.
2. Glycoprotein oligosaccharide analysis and reconstruction of glycan-profiles (various figs.): refer to publication for details.