

For analysis of various ER proteins



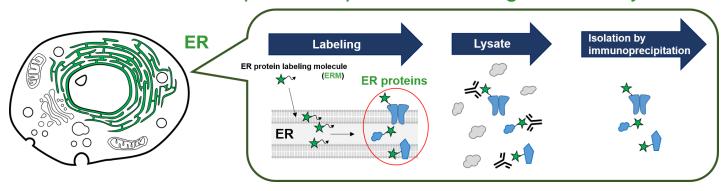
ER-Protein Capture Kit

For more information: https://www.funakoshi.co.jp/exports_contents/81520

ER (Endoplasmic reticulum) analysis is presently limited because current isolation methods are low selectively...



Enables to capture ER proteins with high selectivity!



ER-Protein Capture Kit specifically labels and enables to isolate ER-associated proteins by immunoprecipitation. This kit is a powerful tool for analyzing global ER-associated proteins with easy procedures and without special equipment such as ultracentrifuge.

Application

- -Comprehensive identification of ER-associated proteins
- -Quantitative profiling of ER associated proteins

▶ Reference

1. Fujisawa *et al., J. Am. Chem. Soc.*, **140**, 17060-17070 (2018)

Chemical Profiling of the Endoplasmic Reticulum Proteome Using Designer Labeling Reagents.

▶ Product information

[Manufacturer : FNA]

Product Name	Code	Size	Storage	Price
ER-Protein Capture Kit	FDV-0039	1 kit	-20°C	

Kit contents: A: ER protein labeling molecule (ERM), B: Antibody for immunoprecipitation

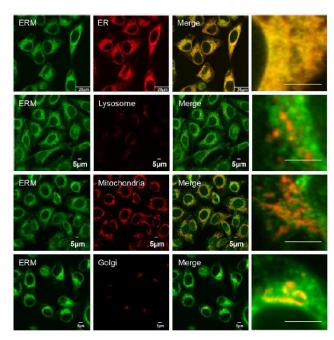
► ER specificity

Hela cells were treated with component A, ERM (100 nM) and organelle markers, Glibenclamide type ER staining, lysosomal staining, mitochondrial staining and Golgi apparatus staining dyes.

ERM was highly overlapped with ER but not correlated with the lysosome marker or mitochondria marker.

Only a small portion of staining by ERM was overlapped with the Golgi apparatus.

It was considered that this is attributed to the vesicle transport of ERM itself or rhodol labeled proteins from ER to the Golgi apparatus.

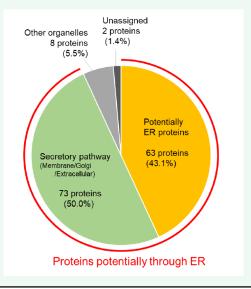


► Application data

Comprehensive identification of ER-associated proteins by LC/MS proteomics

HeLa cells were treated with 100 nM ERM and lysed by cell lysis buffer. ERM-tagged proteins are selectively purified by immunoprecipitation with the antibody and Protein A-beads. Purified proteins are separated by SDS-PAGE and analyzed by LC/MS-based proteomics.

Total of 146 proteins were identified in this experiment. 63 proteins were categorized in ER resident proteins and 73 proteins were categorized in the secretory pathways such as membrane, Golgi apparatus, and extracellular proteins. As secretory pathway proteins basically move to the final destination via ER, ERM based assay could identify ER associated proteins with around 93% probability.



Quantitative profiling of ER associated proteins during ER stress by SILAC assay

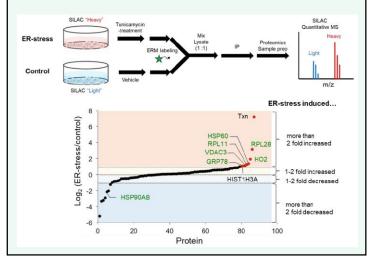
HeLa cells were continuously grown in "light" SILAC media or "heavy" SILAC media.

The "heavy" isotope labeled cells and the "light" isotope labeled cells were treated with tunicamycin, a chemical inducer of ER stress for 4 hours, respectively.

After the treatment, both cells were incubated with 100 nM ERM for 1 hour and lysed by cell lysis buffer.

Equal amounts of "heavy" isotope labeled proteins and "light" isotope labeled proteins were mixed in a 1:1 ratio and ERM labeled proteins were purified with the antibody /protein A beads and analyzed.

A total of 87 proteins were identified and quantified. SILAC analysis indicates 6 proteins were upregulated by more than 2 fold in tunicamycin treated cells.



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