#12718 Store at -20°C

ER and Golgi-Associated Marker Proteins Antibody Sampler Kit

☑ 1 Kit
(5 x 20 µl)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Calnexin (C5C9) Rabbit mAb	2679	20 µl	90 kDa	Rabbit IgG
ERp72 (D70D12) XP® Rabbit mAb	5033	20 µl	72 kDa	Rabbit IgG
PDI (C81H6) Rabbit mAb	3501	20 µl	57 kDa	Rabbit IgG
RCAS1 (D2B6N) XP® Rabbit mAb	12290	20 µl	32 kDa	Rabbit IgG
Syntaxin 6 (C34B2) Rabbit mAb	2869	20 µl	32 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The ER and Golgi-Associated Marker Proteins Antibody Sampler Kit contains reagents to examine proteins that help regulate protein folding and vesicle trafficking. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Secretory and transmembrane proteins are synthesized on polysomes and translocate into the endoplasmic reticulum (ER) where they are often modified by the formation of disulfide bonds, amino-linked glycosylation and folding. The ER contains a pool of molecular chaperones to help proteins fold properly. Calnexin is a calcium-binding, ER membrane protein that ensures proper protein folding by retaining newly synthesized glycoproteins within the ER l (1-3). The specificity of calnexin for a subset of glycoproteins is defined by a lectin site, which binds an early oligosaccharide intermediate on the folding glycoprotein (3). Many secretory proteins require the formation of intra- or inter-molecular disulfide bonds to reach their native conformation (4). Protein disulfide isomerase (PDI) catalyzes the formation and isomerization of disulfide bonds during oxidative protein folding (5). The ER-protein Ero1 oxidizes PDI through disulfide exchange, which is followed by PDI-catalyzed disulfide bond formation in folding proteins (6). The ER stress protein 72 (ERp72) contains three thioredoxin homology domains and plays a role in the formation and isomerization of disulfide bonds (7,8).

The tumor-associated antigen RCAS1 negatively regulates cytotoxic T lymphocyte (CTL) cytolytic activity, which impacts vesicle formation, secretion, and protein glycosylation (9-12). Overexpression of RCAS1 impairs CTL cytolytic function by negatively regulating trans-Golgi to secretory lysosome protein trafficking, leading to a delay in ER to Golgi vesicle transport and mislocalization of ER quality control and glycosylation proteins. As a result, RCAS1 induces deposition of tumor-associated glycan antigens on the cell surface, which may contribute to tumor pathogenesis through the mediation of adhesion, invasion, and metastasis (13,14). Syntaxin 6 is a ubiquitously expressed S25C family member of the SNARE proteins (15,16) that is localized to the trans-Golgi and within endosomes. It regulates membrane trafficking by partnering with a variety of other SNARE proteins (17-19) and is involved in the regulation of GLUT4 trafficking, neutrophil exocytosis and granule secretion (20-22).

Specificity/Sensitivity: Each antibody will detect endogenous total levels of their target protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence of human calnexin, the sequence of human PDI, the residues surrounding Met279 of human ERp72 protein, the residues surrounding Gly147 of human RCAS1 protein, and the residues surrounding Tyr140 of mouse syntaxin 6 protein.

Background References:

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(3) Williams, D.B. (2006) *J Cell Sci* 119, 615-23.
(4) Huppa, J.B. and Ploegh, H.L. (1998) *Cell* 92, 145-8.
(5) Ellgaard, L. and Ruddock, L.W. (2005) *EMBO Rep* 6, 28-32.
(6) Tu, B.P. and Weissman, J.S. (2004) *J Cell Biol* 164, 341-6.
(7) Mazzarella, RA et al. (1990) *J Biol Chem* 265(2), 1094-101.
(8) Satoh, M et al. (2005) *Cell Stress Chaperones* 10(4), 278-84
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(10) Reimer, T.A. et al. (2005) *BMC Cancer* 5, 47.
(11) Wolf, J. et al. (2010) *FASEB J* 24, 4000-19.
(12) Engelsberg, A. et al. (2003) *J Biol Chem* 278, 22998-3007.
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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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#12718

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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