

Technical Data Sheet

Purified Mouse Anti-Calnexin

Product Information

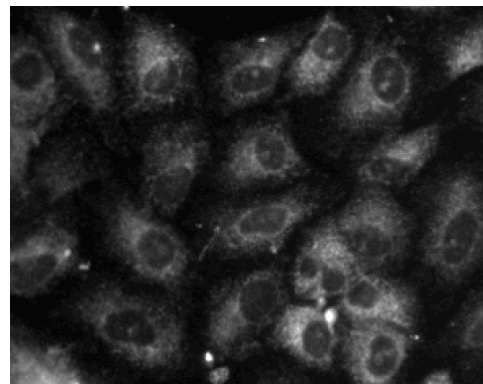
Material Number:	610523
Size:	50 µg
Concentration:	250 µg/ml
Clone:	37/Calnexin
Immunogen:	Human Calnexin aa. 116-301
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Dog
Target MW:	90 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Calnexin is a 90 kDa integral membrane protein located primarily in the ER. It contains a long N-terminal calcium-binding domain that extends into the lumen of the ER and a short, acidic cytosolic domain. Calnexin associates with several cell surface proteins as they pass through the ER. Since it also forms complexes with other resident ER proteins involved in the Ca²⁺-dependent retention of proteins, calnexin may act as a chaperone. The amino acid sequence of calnexin is highly conserved among species and is similar in sequence to calreticulin, another Ca²⁺-binding protein found in the ER. Neither calnexin nor calreticulin contain the calcium-binding motif, known as the "E-F hand", found in the calmodulin family of proteins.



Western blot analysis for Calnexin on a HeLa cell lysate (Human cervical epitheloid carcinoma; ATCC CCL-2). Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the Mouse Anti-Calnexin antibody.



Immunofluorescence staining of A549 (Human lung carcinoma; ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10 000 cells per well. After overnight incubation, cells were stained using the Perm Buffer III protocol and the Mouse Anti-Calnexin antibody. The second step reagent was a FITC Goat Anti-Mouse Ig polyclonal antibody (Cat. No. 554001). Images were taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells with either the Triton™ X-100 and or Perm Buffer III protocols (see Recommended Assay Procedure).

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/certified_reagents.jsp. An abbreviated protocol is listed below:

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Phosflow™ Perm Buffer III, 90% methanol or Triton™ X-100:
 - a. Add 100 µl of -20°C chilled Perm Buffer III (Cat. No. 558050) or 90% methanol to each well and incubate for 5 minutes at RT.
- OR
 - b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611449	HeLa Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Triton is a trademark of the Dow Chemical Company.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.