Human/Mouse/Rat Calreticulin Antibody

Monoclonal Mouse IgG_{2B} Clone # 681233

Catalog Number: MAB38981

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human Calreticulin in ELISA. Detects human, mouse and rat Calreticulin in Western blots.
Source	Monoclonal Mouse IgG_{2B} Clone # 681233
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human Calreticulin Glu18-Leu417 Accession # P27797
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

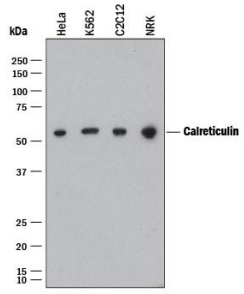
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Knockout Validated	Calreticulin is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in Calreticulin knockout HeLa cell line.	

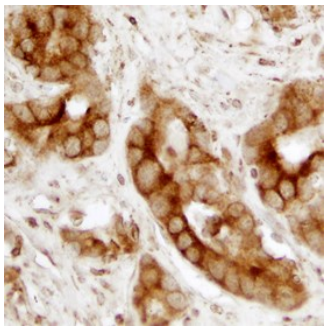
DATA

Western Blot



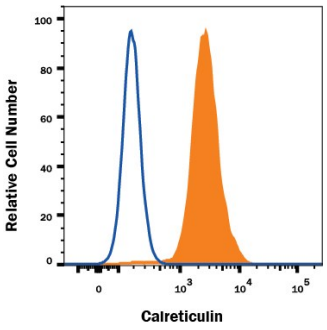
Detection of Human, Mouse, and Rat Calreticulin by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, K562 human chronic myelogenous leukemia cell line, C2C12 mouse myoblast cell line, and NRK rat normal kidney cell line. PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human Calreticulin Monoclonal Antibody (Catalog # MAB38981) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Calreticulin at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



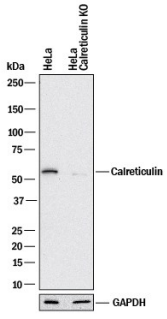
Calreticulin in Human Prostate. Calreticulin was detected in formalin fixed paraffin-embedded sections of human prostate using Mouse Anti-Human Calreticulin Monoclonal Antibody (Catalog # MAB38981) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membrane and cytoplasm. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Intracellular Staining by Flow Cytometry



Detection of Calreticulin in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Mouse Anti-Human Calreticulin Monoclonal Antibody (Catalog # MAB38981, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for Staining Intracellular Molecules.

Knockout Validated



Western Blot Shows Human Calreticulin Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and Calreticulin knockout HeLa cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human/Mouse/Rat Calreticulin Monoclonal Antibody (Catalog # MAB38981) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Calreticulin at approximately 55 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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Human/Mouse/Rat Calreticulin Antibody

Monoclonal Mouse IgG_{2B} Clone # 681233

Catalog Number: MAB38981

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Human Calreticulin is a 55-60 kDa, 400 amino acid, variably glycosylated intra- and extracellular Ca^{++}-binding lectin that is ubiquitously expressed. It consists of three domains: a 180 aa N-terminal globular region, a 111 aa P-, or proline rich domain, and a 109 aa C-terminus. The 180 aa N-terminus (aa 18-197) is termed Vasostatin. It is unclear if it is ever generated naturally via proteolytic processing. Vasostatin domain has many functions. It binds to RNA (aa 18-27), has autocatalytic phosphorylase activity (aa 77-197), binds to a KxFFKR motif on steroid hormone receptors, and serves as a lectin-type chaperone for ER-localized molecules. It also shows antiangiogenic activity, presumably by binding to laminin carbohydrates and blocking endothelial cell adhesion and proliferation. Human Calreticulin is 94% aa identical to mouse and rat Calreticulin.

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