

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Cathepsin D in direct ELISAs and Western blots. It recognizes both the pro and mature forms of recombinant human (rh) Cathepsin D. In direct ELISAs, no cross-reactivity with rhCathepsin A, rhCathepsin B, rhCathepsin C, rhCathepsin L, rhCathepsin O, rhCathepsin S, rhCathepsin Z, or recombinant mouse Cathepsin D is observed. In Western blots, 100% cross-reactivity with rhCathepsin E and rmCathepsin D is observed and no cross-reactivity with rhBACE-1 is observed.
Source	Monoclonal Mouse IgG ₁ Clone # 185111
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Cathepsin D Leu21-Leu412 Accession # P07339
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 as a 0.2 µm filtered solution in PBS.

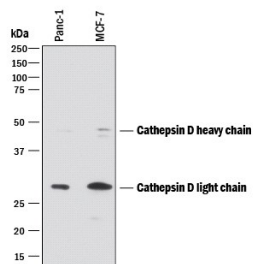
APPLICATIONS

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	0.2 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
Simple Western	2 µg/mL	See Below

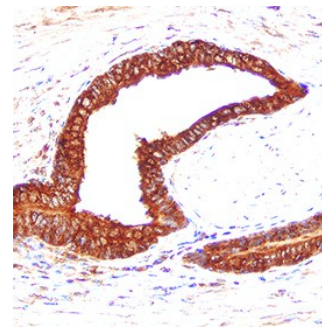
DATA

Western Blot



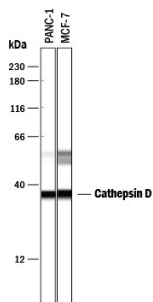
Detection of Human Cathepsin D by Western Blot. Western blot shows lysates of PANC-1 human pancreatic carcinoma cell line and MCF-7 human breast cancer cell line. PVDF membrane was probed with 0.2 µg/mL of Mouse Anti-Human Cathepsin D Monoclonal Antibody (Catalog # MAB1014) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). Specific bands were detected for Cathepsin D at approximately 28 and 46 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



Cathepsin D in Human Prostate Cancer Tissue. Cathepsin D was detected in immersion fixed paraffin-embedded sections of human prostate cancer tissue using Mouse Anti-Human Cathepsin D Monoclonal Antibody (Catalog # MAB1014) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to epithelial cell cytoplasm. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human Cathepsin D by Simple Western™. Simple Western lane view shows lysates of PANC-1 human pancreatic carcinoma cell line and MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. Specific bands were detected for Cathepsin D at approximately 36 and 52-57 kDa (as indicated) using 2 µg/mL of Mouse Anti-Human Cathepsin D Monoclonal Antibody (Catalog # MAB1014). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 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

Cathepsin D is a lysosomal aspartic protease of the pepsin family (1). Human cathepsin D is synthesized as a precursor protein, consisting of a signal peptide (aa 1-18), a propeptide (aa 19-64), and a mature chain (aa 65-412) (2-4). The mature chain can be processed further to the light (aa 65-161) and heavy (aa 169-412) chains. It is expressed in most cells and overexpressed in breast cancer cells (5). It is a major enzyme in protein degradation in lysosomes, and also involved in the presentation of antigenic peptides. Mice deficient in this enzyme showed a progressive atrophy of the intestinal mucosa, a massive destruction of lymphoid organs, and a profound neuronal ceroid lipofucinosi, indicating that cathepsin D is essential for proteolysis of proteins regulating cell growth and tissue homeostasis (6). Cathepsin D secreted from human prostate carcinoma cells are responsible for the generation of angiostatin, a potent endogeneous inhibitor of angiogenesis (6).

References:

1. Conner *et al.* in *Handbook of Proteolytic Enzymes* Barrett (1998) Academic Press, San Diego, p. 828.
2. Faust, *et al.* (1985) *Proc. Natl. Acad. Sci. USA* **82**:4910.
3. Westley and May (1987) *Nucl. Acid Res.* **15**:3773.
4. Redecker, *et al.* (1991) *DNA Cell Biol.* **10**:423.
5. Rochefort, *et al.* (2000) *Clin. Chim. Acta.* **291**:157.
6. Tsukuba, *et al.* (2000) *Mol. Cells* **10**:601.