

DESCRIPTION

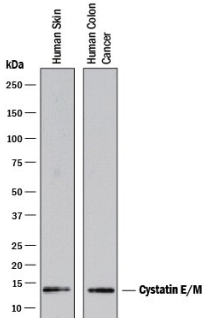
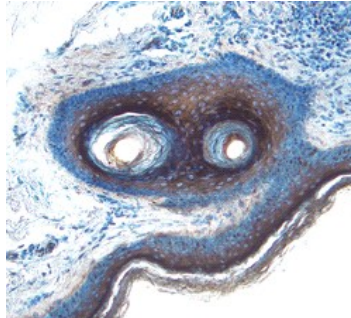
Species Reactivity	Human
Specificity	Detects human Cystatin E/M in direct ELISAs and Western blots. In direct ELISAs and Western blots, no cross-reactivity with recombinant human (rh) Cystatins A, B, C, S, SA, SN, rhFetuin A, B, rhHRGP, or rhKininogen is observed.
Source	Monoclonal Mouse IgG _{2B} Clone # 211515
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Cystatin E/M Arg29-Met149 Accession # Q15828
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	2 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Immunoprecipitation	25 µg/mL	Conditioned cell culture medium spiked with Recombinant Human Cystatin E/M (Catalog # 1286-PI), see our available Western blot detection antibodies
Neutralization		Measured by its ability to neutralize Recombinant Human Cystatin E/M (0.32 µg/mL, Catalog # 1286-PI) inhibition of Papain (0.1 µg/mL) cleavage of the fluorogenic peptide substrate Z-FR-AMC (100 µM, Catalog # ES009). The Neutralization Dose (ND ₅₀) is typically 7 µg/mL.

DATA

<p>Western Blot</p>  <p>Detection of Human Cystatin E/M by Western Blot. Western blot shows lysates of human skin tissue and human colon cancer tissue. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human Cystatin E/M Monoclonal Antibody (Catalog # MAB1286) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Cystatin E/M at approximately 13 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunohistochemistry</p>  <p>Cystatin E/M in Human Skin. Cystatin E/M was detected in immersion fixed paraffin-embedded sections of human skin using 25 µg/mL Mouse Anti-Human Cystatin E/M Monoclonal Antibody (Catalog # MAB1286) overnight at 4 °C. Tissue was stained with the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific labeling was localized to the cytoplasm of cells in hair follicles. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>
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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. <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

Cystatin E/M encoded by the CST6 gene is a member of family 2 of the cystatin superfamily (1, 2). It inhibits papain and cathepsin B, two of the cysteine proteases. Its mRNA was found in many tissues by the two groups who did initial cloning (1, 2). However, its protein was found only in skin and sweat glands by a third group (3). In addition to being a cysteine protease inhibitor, Cystatin E/M is also a substrate for transglutaminases (3). It is required for viability and for correct formation of cornified layers in the epidermis and hair follicles, as *ichq* mice, with a null mutation in the Cystatin E/M gene, have defects in epidermal cornification and die between 5 and 12 days of age (4). Cystatin E/M expression and function may not be limited to cutaneous epithelia. For example, it is found in rat brain and is induced during neuronal cell differentiation (5).

References:

1. Sotiropoulou, G. *et al.* (1997) *J. Biol. Chem.* **272**:903.
2. Ni, J. *et al.* (1997) *J. Biol. Chem.* **272**:10853.
3. Zeeuwen, P.L. *et al.* (2001) *J. Invest. Dermatol.* **116**:693.
4. Zeeuwen, P.L. *et al.* (2002) *Hum. Mol. Genet.* **11**:2867.
5. Hong, J. *et al.* (2002) *J. Neurochem.* **81**:922.