

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human ELA2 in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG ₁ Clone # 950317
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human ELA2 Met1-Asn252 Accession # P08246
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	0.1 µg/mL	See Below
Immunocytochemistry	5-25 µg/mL	See Below
Immunohistochemistry	3-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

DATA

Western Blot

Detection of Human Neutrophil Elastase/ELA2 by Western Blot. Western blot shows lysates of human bone marrow and human lung tissue. PVDF membrane was probed with 0.1 µg/mL of Mouse Anti-Human Neutrophil Elastase/ELA2 Monoclonal Antibody (Catalog # MAB91671) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). Specific bands were detected for Neutrophil Elastase/ELA2 at approximately 25-30 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry

Neutrophil Elastase/ELA2 in THP-1 Human Cell Line. Neutrophil Elastase/ELA2 was detected in immersion fixed THP-1 human acute monocytic leukemia cell line using Mouse Anti-Human Neutrophil Elastase/ELA2 Monoclonal Antibody (Catalog # MAB91671) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry

Neutrophil Elastase/ELA2 in Human Lymphoma. Neutrophil Elastase/ELA2 was detected in immersion fixed paraffin-embedded sections of human lymphoma using Mouse Anti-Human Neutrophil Elastase/ELA2 Monoclonal Antibody (Catalog # MAB91671) 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 cytoplasm. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Intracellular Staining by Flow Cytometry

Detection of Neutrophil Elastase/ELA2 in THP-1 Human Cell Line by Flow Cytometry. THP-1 human acute monocytic leukemia cell line treated with 3 µM monensin for 3 hours was stained with Mouse Anti-Human Neutrophil Elastase/ELA2 Monoclonal Antibody (Catalog # MAB91671, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). 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.

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

Neutrophil Elastase (ELA2, ELANE), also known as HNE, is a chymotrypsin family serine protease that plays a key role in pathogen clearance (1-3). It is expressed by promyelocytes and stored in the intracellular azurophilic granules of polymorphonuclear leukocytes (PMN) (4). These granules fuse with phagosomes, enabling Neutrophil Elastase to participate in the digestion and killing of endocytosed microbes. The enzyme is released by activated neutrophils at sites of inflammation, and it can remain associated with the cell surface or function as a component of neutrophil extracellular nets (NETs) which trap and kill microbial pathogens (5, 6). It also can degrade multiple extracellular matrix proteins including Elastin and Fibronectin (5). In the lung, this activity contributes to pathology in emphysema, cystic fibrosis, and adult respiratory distress syndrome (ARDS) (1). Neutrophil Elastase can be inhibited by Serpin A1/alpha 1-Antitrypsin, SLPI, Serpin B1, and Trappin-2/Elafin (7-11). Its activity in the lung is increased by exposure to tobacco smoke which inactivates Serpin A1 through methionine oxidation (12). Mature human Neutrophil Elastase shares 73% amino acid sequence identity with mouse and rat Neutrophil Elastase (13, 14). Multiple mutations in the human ELANE gene are causative of severe congenital and cyclic neutropenias (15).

References:

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