

NETosis Imaging and Flow Assay Kit

Catalog# / Size	421952 / 1 kit
Regulatory Status	RUO
Other Names	Netosis, elastase
Description	The NETosis Imaging and Flow Assay Kit is designed for the quantification and analysis of NETosis in both human and murine neutrophils by flow cytometry and live cell imaging. NETosis is a specialized immune response in which neutrophils release neutrophil extracellular traps (NETs)—web-like chromatin structures coated with antimicrobial proteins and proteases such as neutrophil elastase—to capture and neutralize pathogens. This kit combines a neutrophil elastase-activated fluorescent probe with two DNA-binding dyes: Hoechst 33342, which is membrane-permeable, and Helix Green, which is membrane-impermeable. These components facilitate the analysis of NETosis by detecting neutrophil elastase activity, extracellular DNA, and intact nuclei, offering a streamlined and rapid method for obtaining reliable results.

Kit Details

Kit Contents	• Elastase Probe Far Red, 1 vial, lyophilized (Ex 675/Em 720, APC filter set) • Hoechst 33342 solution, 1 vial, 150 µL (Ex 350/Em 461, DAPI filter set) • Helix Green, 1 vial, 250 µL (Ex 495/Em 519, FITC filter set) • PMA, 0.1mg/mL in DMSO, 1 vial, 200 µL
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Product Details

Verified Reactivity	Human, Mouse
Storage & Handling	-20°C, protected from light
Application	FC - Quality tested Live cell imaging - Verified In vivo imaging - Reported in the literature, not verified in house
Recommended Usage	Refer to product manual
Application References	1. Scales HE, <i>et al.</i> 2016. <i>Rheumatology</i>. 55(3):564-72. 2. Kolaczowska E, <i>et al.</i> 2015. <i>Nat Commun</i>. 6, 6673. 3. Mitra S, <i>et al.</i> 2013. <i>J Biomed Opt</i>. 18(10):101314.
(PubMed link indicates BioLegend citation)	

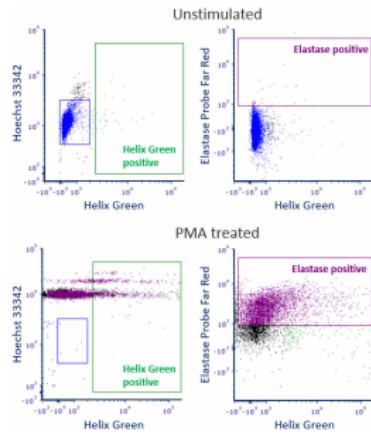
Antigen Details

Cell Type	Granulocytes, Neutrophils
Biology Area	Apoptosis/Tumor Suppressors/Cell Death, Cell Biology, Cell Death, Cell Motility/Cytoskeleton/Structure, Cell Structure, Immunology, Innate Immunity
Molecular Family	Enzymes and Regulators, Innate Immune Signaling, Nuclear Markers, Organelle Markers
Gene ID	NA

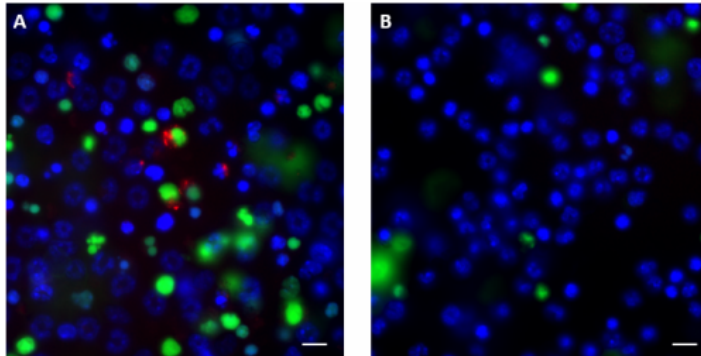
Related Protocols

- [Cell Surface Flow Cytometry Staining Protocol](#)

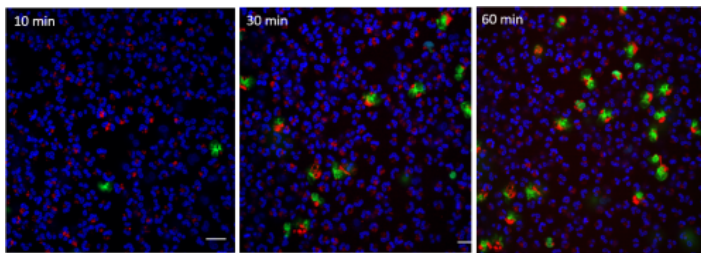
Product Data



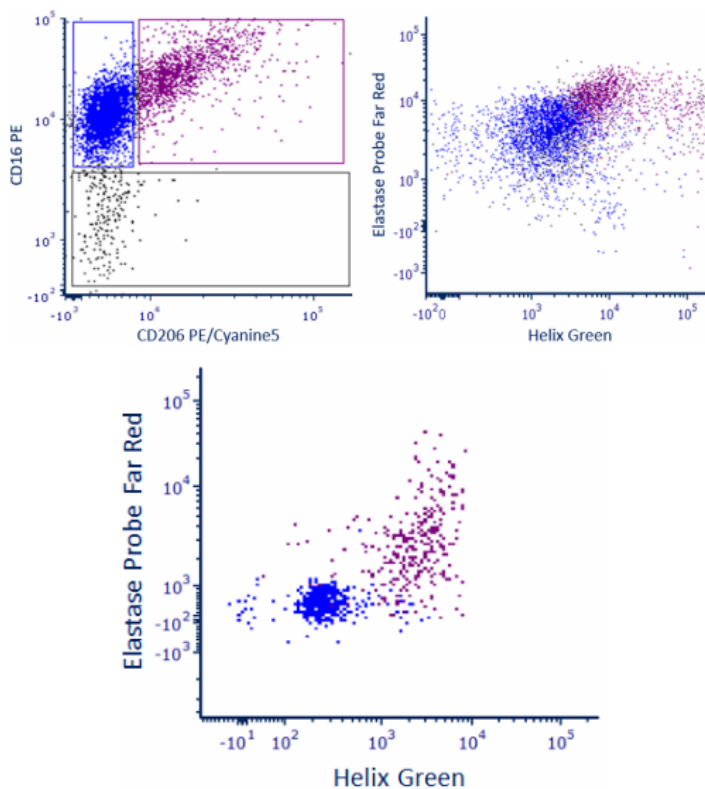
Human peripheral blood granulocytes were resuspended in RPMI media supplemented with 10% fetal bovine serum and treated with PMA (bottom row) or left untreated (top row) for two hours at 37°C. Cells were then stained using the NETosis Imaging and Flow Assay Kit. Cells were fixed prior to analysis. Unstimulated granulocytes have intact nuclei (blue) and are elastase-negative. PMA-treated granulocytes that have undergone NETosis are Helix Green- and elastase-positive (purple) and lack intact nuclei.



Neutrophils were isolated from C57BL/6 mouse bone marrow using the MojoSort™ Mouse Neutrophil Isolation Kit (Cat. No. 480058). Cells were resuspended in RPMI media supplemented with 10% fetal bovine serum and treated with Cell Activation Cocktail (panel A) or left unstimulated (panel B) for 4 hours. Cells were then stained using the NETosis Imaging and Flow Assay Kit and protocol: Hoechst 33342 (blue), Helix Green (green), Elastase probe (red). Helix Green positive NETs are elastase positive, negative for Hoechst (live DNA). Cells were imaged using a 63X objective. Scale bar = 20µm



Human peripheral blood granulocytes were isolated by density gradient centrifugation. Cells were resuspended in phenol-free media supplemented with 10% fetal bovine serum and stained using the NETosis Imaging and Flow Assay Kit and protocol: Hoechst 33342 (blue), Helix Green (green), Elastase probe (red). PMA was added to cells and images were taken over time using a spinning-disk confocal microscope equipped with environmental controls and a 40X objective. NETs are Helix Green and elastase positive and negative for Hoechst 33342 (blue). Scale bar 20µm



Human peripheral blood granulocytes were treated with PMA for two hours at 37°C. Cells were then stained using the NETosis Imaging and Flow Assay Kit and protocol and co-stained with anti-human CD16 (clone 3G8) PE and anti-human CD206 (clone 15-2) PE/Cyanine5. In the right plot, CD16+CD206+ N2 neutrophils are shown in purple and CD16+ CD206- N1 neutrophils are shown in blue.

Human peripheral blood granulocytes were incubated with (purple) or without PMA (blue) for two hours at 37°C. Cells were then stained using the NETosis Imaging and Flow Assay Kit and anti-human CD16 (clone 3G8) PE. Data shown are gated on CD16-negative eosinophils.

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