

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Laminin α 3/Laminin-5 in direct ELISAs and Western blots. In direct ELISAs, no cross reactivity with recombinant human (rh) Laminin α 1, β 1, γ 2, or recombinant mouse Laminin α 4 is observed.
Source	Monoclonal Mouse IgG ₁ Clone # 546215
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human Laminin α 3/Laminin-5 aa 21-1713 Accession # NP_000218
Formulation	Lyophilized from a 0.2 μ m 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 μ m filtered solution in PBS.

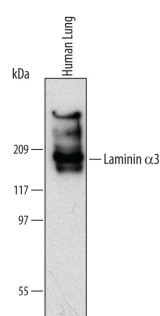
APPLICATIONS

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 μ g/mL	See Below
Immunocytochemistry	8-25 μ g/mL	See Below
Immunohistochemistry	5 μ g/mL	See Below
Immunoprecipitation	25 μ g/mL	Conditioned cell culture medium spiked with Recombinant Human Laminin α 3, see our available Western blot detection antibodies
Intracellular Staining by Flow Cytometry	0.25 μ g/10 ⁶ cells	See Below

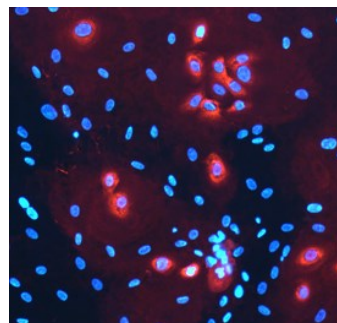
DATA

Western Blot



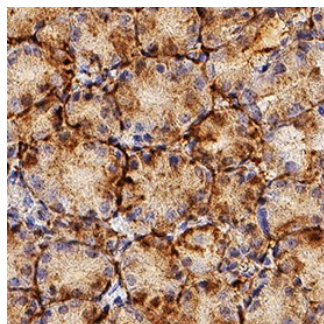
Detection of Human Laminin α 3/Laminin-5 by Western Blot. Western blot shows lysates of human lung tissue. PVDF Membrane was probed with 2 μ g/mL of Mouse Anti-Human Laminin α 3/Laminin-5 Monoclonal Antibody (Catalog # MAB21441) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Laminin α 3/Laminin-5 at approximately 190 kDa (as indicated). This experiment was conducted under non-reducing conditions and using [Immunoblot Buffer Group 1](#).

Immunocytochemistry



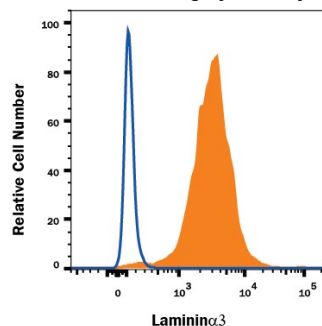
Laminin α 3/Laminin-5 in NHEK Human Cell Line. Laminin α 3/Laminin-5 was detected in immersion fixed NHEK human normal epidermal keratinocytes treated with 10 ng/mL Recombinant Human TGF- β 1 (Catalog # 240-B) for 24 hours using Mouse Anti-Human Laminin α 3/Laminin-5 Monoclonal Antibody (Catalog # MAB21441) at 10 μ g/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 the perinuclear space. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Laminin α 3/Laminin-5 in Human Stomach. Laminin α 3/Laminin-5 was detected in immersion fixed paraffin-embedded sections of human stomach using Mouse Anti-Human Laminin α 3/Laminin-5 Monoclonal Antibody (Catalog # MAB21441) at 5 μ g/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). 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 DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell surface of epithelial cells in gastric glands. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Intracellular Staining by Flow Cytometry



Detection of Laminin α 3/Laminin-5 in U2OS Human Cell Line by Flow Cytometry. U2OS human osteosarcoma cell line was stained with Mouse Anti-Human Laminin α 3/Laminin-5 Monoclonal Antibody (Catalog # MAB21441, filled histogram) or isotype control antibody (Catalog # MAB002, 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](#).

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

Laminins are heterotrimeric, noncollagenous glycoproteins composed of α , β , and γ chains. Through interactions with integrins, dystroglycan and other receptors, laminins contribute to cell differentiation, cell shape and migration, and maintenance of tissue phenotypes and survival. Laminin α 3/Laminin-5, also known as epiligrin, includes α 3, β 3, and γ 2 subunits. It is abundant in transitional epithelium, stratified squamous epithelia, lung mucosa and other epithelial glands and contributes to initiation and maintenance of epithelial cell anchorage to the underlying connective tissue. Within aa 21-1713 of the α 3 subunit, human and mouse share 77% amino acid sequence identity.