

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human HIST3H3 in Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2128A
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human HIST3H3 synthetic peptide
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	0.3-25 µg/mL	See Below
Immunohistochemistry	3-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below

DATA

Western Blot

Detection of Human HIST3H3 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with Sodium (Na) Butyrate. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human HIST3H3 Monoclonal Antibody (Catalog # MAB9448) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for HIST3H3 at approximately 16 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry

HIST3H3 in HeLa Human Cell Line. HIST3H3 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Rabbit Anti-Human HIST3H3 Monoclonal Antibody (Catalog # MAB9448) at 0.3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (yellow; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cell nuclei. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry

HIST3H3 in Human Testis. HIST3H3 was detected in immersion fixed paraffin-embedded sections of human testis using Rabbit Anti-Human HIST3H3 Monoclonal Antibody (Catalog # MAB9448) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell nuclei. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Intracellular Staining by Flow Cytometry

Detection of HIST3H3 in HeLa cells by Flow Cytometry. HeLa human cell line was stained with Rabbit anti-Human HIST3H3 Monoclonal Antibody (Catalog # MAB9448, filled histogram) or Normal Rabbit IgG control (Catalog # MAB1050, open histogram) followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012).

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

Histones are alkaline proteins which organize nuclear DNA into nucleosomes. Histone H3 is one of four core histones, which exist as dimers to form an octameric histone core. Epigenetic modification of the exposed histone tails by methylation or acetylation is known to regulate transcription in various ways and is known to affect the etiology of a variety of cancers and developmental defects. Methylation by methyltransferases mediates transcriptional activation (SET1, Chd1), elongation (MLL, WDR5) or silencing (SET7/9, JMJD2A). Expression of H3K4 methyltransferases have been associated with solid tumors such as prostate, ovary, squamous cell carcinoma, hepatocellular carcinoma and cancers of the blood, including T-cell acute lymphoblastic leukemia, acute myeloid leukemia, acute lymphoblastic leukemia, and others.