

# Histone H3.3 Polyclonal Antibody

Product Details	
Size	100 µL
Species Reactivity	Human, Mouse, Rat
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Conjugate	Unconjugated
Immunogen	Full length human Histone H3.3B Recombinant protein.
Form	Liquid
Concentration	0.16 mg/mL
Purification	Antigen affinity chromatography
Storage buffer	PBS, pH 7, with 20% glycerol, 1% BSA
Contains	0.025% ProClin 300
Storage conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.
RRID	AB_11156178

Applications	Tested Dilution	Publications
Western Blot (WB)	1:500-1:20,000	-
Immunohistochemistry (Paraffin) (IHC (P))	1:100-1:1,000	-
Immunocytochemistry (ICC/IF)	1:100-1:1,000	-
ChIP assay (ChIP)	1.5 µg/1x10^6 cells	-

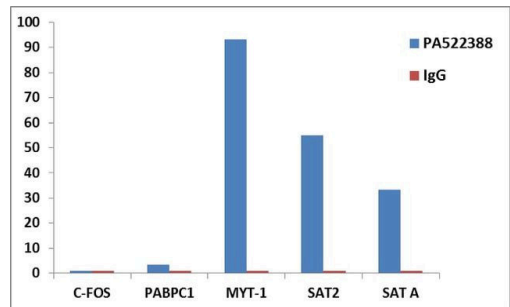
## Product Specific Information

Recommended positive controls: 293T, A431, HeLa, HepG2, Mouse brain, rat brain.

Predicted reactivity: Mouse (100%), Rat (100%), Xenopus laevis (100%), Chicken (100%), Chimpanzee (100%), Bovine (100%).

Store product as a concentrated solution. Centrifuge briefly prior to opening the vial.

Product Images For Histone H3.3 Polyclonal Antibody

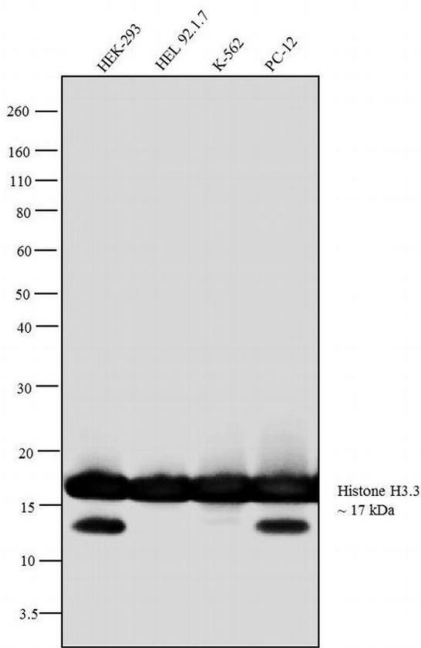


Histone H3.3 Antibody (PA5-22388)

Antibody specificity was demonstrated through detection of enrichment of the target protein at specific gene loci. Chromatin Immunoprecipitation (ChIP) was performed using anti-Histone H3.3B polyclonal antibody (Catalog# PA5-22388) with relevant positive and negative target genes/ binding sites. {RE}

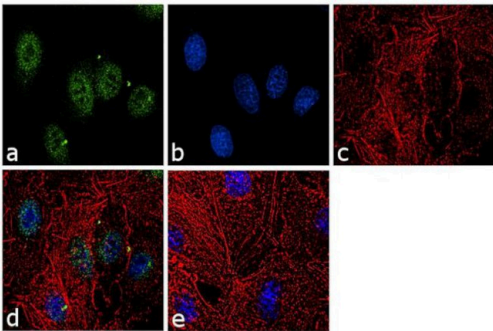
Histone H3.3 Antibody (PA5-22388) in WB

Western blot analysis was performed on acid extracts (20µg lysate) of HEK-293 (Lane 1), HEL 92.1.7 (Lane 2), K-562 (Lane 3) and PC-12 (Lane 4). The blot was probed with Anti-ANTI-Histone H3.3 Rabbit Polyclonal Antibody (Product # PA5-22388, 1:1000 dilution) and detected by chemiluminescence using Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.25µg/mL, 1:4000 dilution). A 17 kDa band corresponding to Histone H3.3 was observed across the cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE®12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



Histone H3.3 Antibody (PA5-22388) in ICC/IF

Immunofluorescence analysis of Histone H3.3 was performed using 70% confluent log phase A549 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Histone H3.3 Rabbit Polyclonal Antibody (Product # PA5-22388) at 2µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing nuclear localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



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