

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human HDAC8 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human HDAC8 isoform 1 Met1-Val377 (Leu31Pro) Accession # Q9BY41
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 as a 0.2 µm filtered solution in PBS.

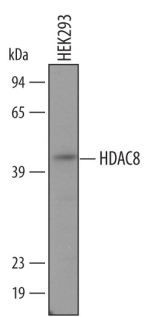
APPLICATIONS

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	1 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/5 x 10 ⁶ cells	See Below

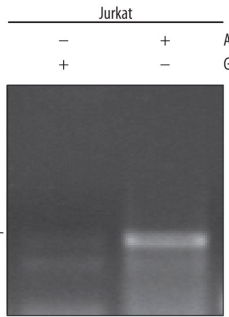
DATA

Western Blot



Detection of Human Histone Deacetylase 8/HDAC8 by Western Blot. Western blot shows lysates of HEK293 human embryonic kidney cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse Histone Deacetylase 8/HDAC8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4359) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Histone Deacetylase 8/HDAC8 at approximately 50 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 8*.

Chromatin Immunoprecipitation (ChIP)



Detection of Histone Deacetylase 8/HDAC8-regulated Genes by Chromatin Immunoprecipitation. Jurkat human acute T cell leukemia cell line treated with 50 ng/mL PMA and 200 ng/mL calcium ionomycin for 30 minutes was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Histone Deacetylase 8/HDAC8/DNA complexes were immunoprecipitated using 5 µg Sheep Anti-Human/Mouse Histone Deacetylase 8/HDAC8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4359) or control antibody (Catalog # 5-001-A) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Sheep IgG Secondary Antibody (Catalog # BAF016). Immunocomplexes were captured using 50 µL of MagCollect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *IFN-γ* promoter was detected by standard PCR.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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

HDAC8 (Histone deacetylase 8) is a 50 kDa member of the class I subfamily, histone deacetylase family of transcriptional regulators. It is a smooth muscle enzyme that deacetylates histone lysines, blocking gene transcription. HDAC8 appears to function as a monomer, and phosphorylation at Ser39 inhibits activity. Human HDAC8 is 377 amino acids (aa) in length. It contains a series of α helices and strands, plus a catalytic ferrous ion binding site (Asp178-His180-Asp267). There are four potential splice variants, all of which possess an alternate start site 38 aa upstream of the standard start site. Three have additional changes; two show a one and three aa substitution respectively for aa 211-377, while a third has a 15 aa substitution for aa 148-377. Human HDAC8 shares more than 96% aa identity with both mouse and canine HDAC8.