

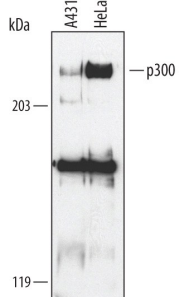
DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human p300 in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human p300 His2283-His2414 Accession # Q09472
Formulation	Lyophilized from a 0.2 µm filtered solution 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
Immunocytochemistry	1-15 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
Simple Western	50 µg/mL	See Below

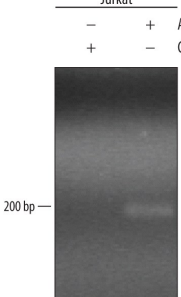
DATA

Western Blot



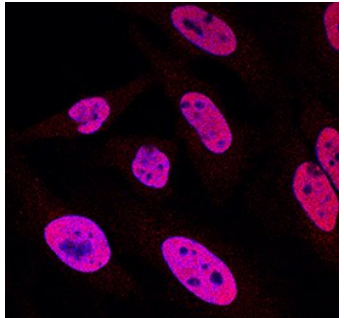
Detection of Human p300 by Western Blot. Western blot shows nuclear extracts of A431 human epithelial carcinoma cell line epidermoid carcinoma and HeLa human cervical epithelial carcinoma cell line cervical carcinoma cell lines. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human p300 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3789) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for p300 at approximately 300 kDa (as indicated). This experiment was conducted using Immunoblot Buffer Group 1.

Chromatin Immunoprecipitation (ChIP)



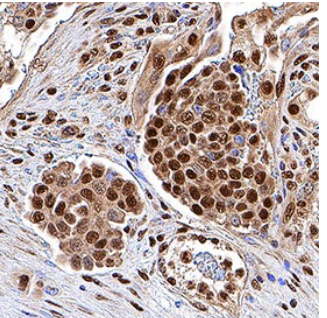
Detection of p300-regulated Genes by Chromatin Immunoprecipitation. Jurkat human acute T cell leukemia cell line treated with 50 ng/mL PMA and 200 ng/mL calcium ionomycin for 30 minutes was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. p300/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human p300 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3789) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCollect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *fos* promoter was detected by standard PCR.

Immunocytochemistry



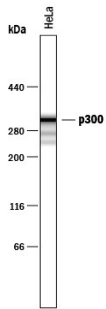
p300 in HeLa Human Cell Line. p300 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human p300 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3789) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



p300 in Human Colon Cancer Tissue. p300 was detected in immersion fixed paraffin-embedded sections of human colon cancer tissue using Goat Anti-Human p300 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3789) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human p300 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for p300 at approximately 308 kDa (as indicated) using 50 µg/mL of Goat Anti-Human p300 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3789) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.



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

The E1A-binding protein p300 is a histone acetyltransferase that also acts to acetylate other proteins, such as p53. p300 acts as a transcriptional coactivator that can serve as an adaptor molecule to bridge to transcriptional regulators. In addition, p300 binds to PCNA and may participate in chromatin remodeling.