10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6)

Catalog # ABO15046

Specification

Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P45973
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) - Additional Information

Gene ID 23468

Other Names
Chromobox protein homolog 5, Antigen p25, Heterochromatin protein 1 homolog alpha, HP1 alpha, CBX5, HP1A

Calculated MW
22 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human, Mouse, Rat
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen
E.coli-derived human HP1 alpha/CBX5 recombinant protein (Position: M1-S191).

Purification
Immunogen affinity purified.

Storage	**Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.**

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Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) - Protein Information

Name CBX5

Synonyms HP1A

Function
Component of heterochromatin that recognizes and binds histone H3 tails methylated at 'Lys-9' (H3K9me), leading to epigenetic repression. In contrast, it is excluded from chromatin when 'Tyr-41' of histone H3 is phosphorylated (H3Y41ph) (PubMed:19783980). May contribute to the association of heterochromatin with the inner nuclear membrane by interactions with the lamin-B receptor (LBR) (PubMed:19783980). Involved in the formation of kinetochore through interaction with the MIS12 complex subunit NSL1 (PubMed:19783980, PubMed:20231385). Required for the formation of the inner centromere (PubMed:20231385).

Cellular Location
Nucleus. Chromosome. Chromosome, centromere. Note=Colocalizes with HNRNPU in the nucleus (PubMed:19617346). Component of centromeric and pericentromeric heterochromatin. Associates with chromosomes during mitosis. Associates specifically with chromatin during metaphase and anaphase (PubMed:19617346). Localizes to sites of DNA damage (PubMed:28977666)

Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) - Images

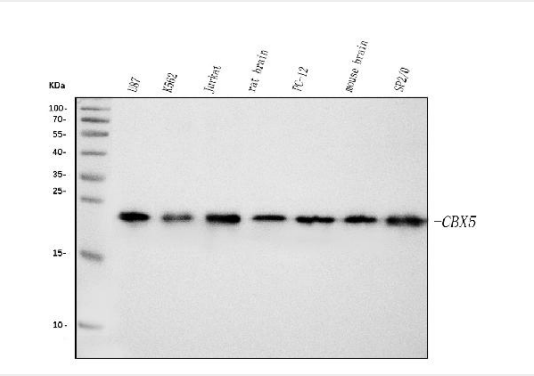

Figure 1. Western blot analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human U87 whole cell lysates,
Lane 2: human K562 whole cell lysates,
Lane 3: human Jurkat whole cell lysates,
Lane 4: rat brain tissue lysates,
Lane 5: rat PC-12 whole cell lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: mouse SP2/0 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-HP1 alpha/CBX5 antigen affinity purified monoclonal antibody (Catalog # M02780-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for HP1 alpha/CBX5 at approximately 22KD. The expected band size for HP1 alpha/CBX5 is at 22KD.

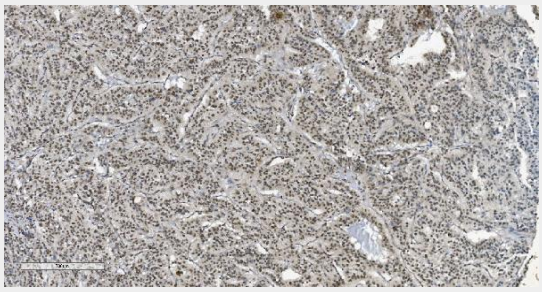

Figure 2. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human adrenocortical adenoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

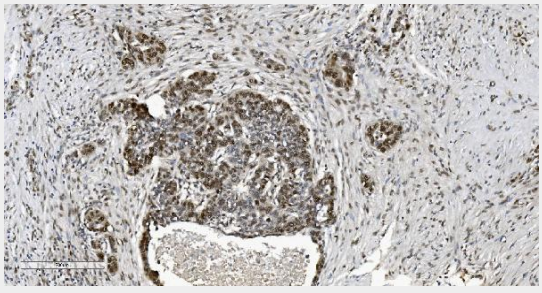

Figure 3. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex

(SABC) (Catalog # SA1021) with DAB as the chromogen.

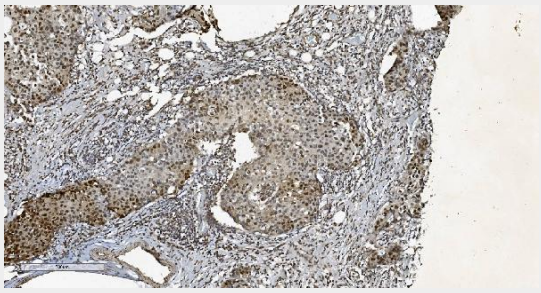

Figure 4. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

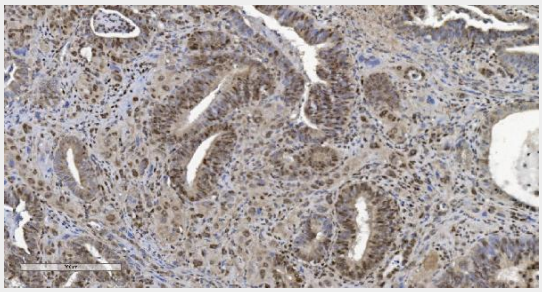

Figure 5. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

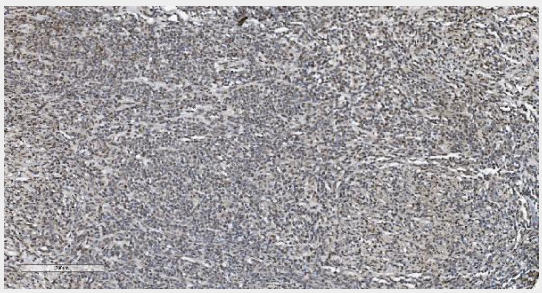

Figure 6. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human lymphoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

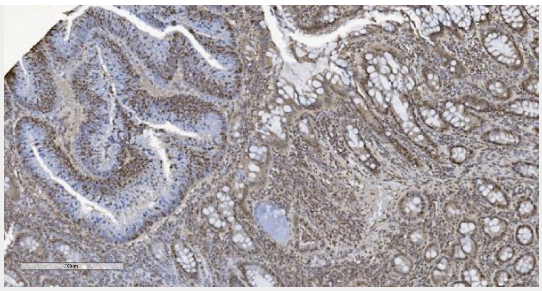

Figure 7. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

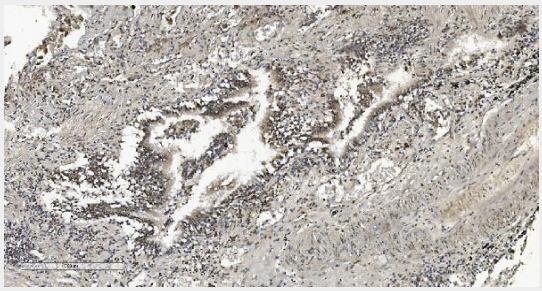

Figure 8. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

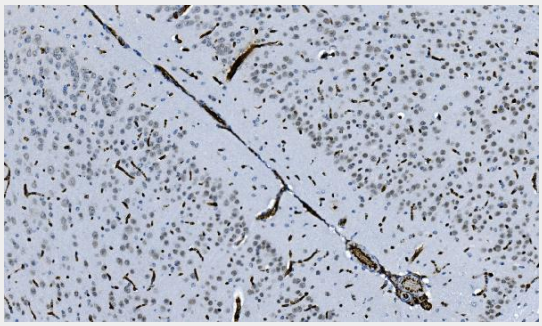

Figure 9. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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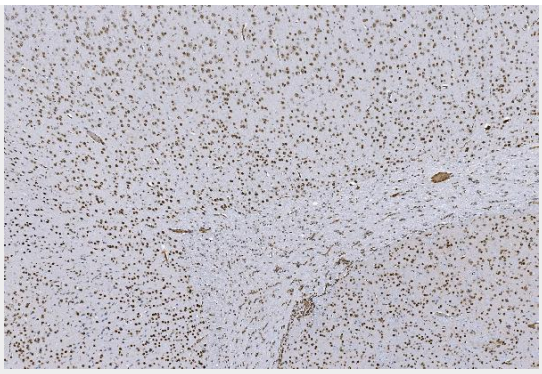

Figure 10. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

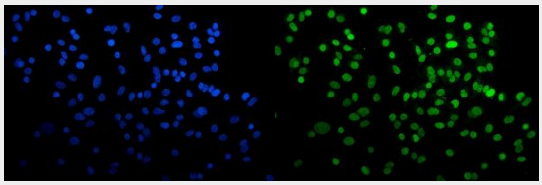

Figure 11. IF analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-HP1 alpha/CBX5 Antibody (M02780-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

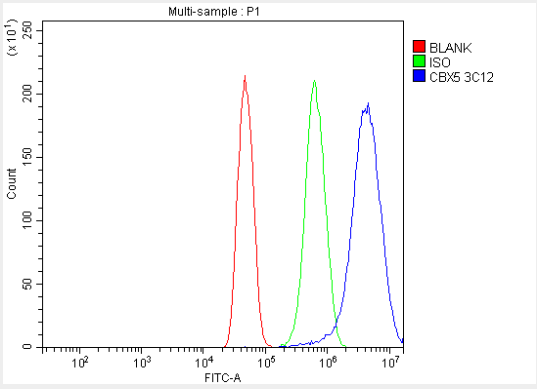

Figure 12. Flow Cytometry analysis of U251 cells using anti-HP1 alpha/CBX5 antibody (M02780-2).
Overlay histogram showing U251 cells stained with M02780-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HP1 alpha/CBX5 Antibody (M02780-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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Anti-HP1 alpha/CBX5 Antibody Picoband™ (monoclonal, 8G6) - Background

This gene encodes a highly conserved nonhistone protein, which is a member of the heterochromatin protein family. The protein is enriched in the heterochromatin and associated with centromeres. The protein has a single N-terminal chromodomain which can bind to histone proteins via methylated lysine residues, and a C-terminal chromo shadow-domain (CSD) which is responsible for the homodimerization and interaction with a number of chromatin-associated nonhistone proteins. The encoded product is involved in the formation of functional kinetochore through interaction with essential kinetochore proteins. The gene has a pseudogene located on chromosome 3. Multiple alternatively spliced variants, encoding the same protein, have been identified.