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). Can interact with lamin-B receptor (LBR). This interaction can contribute to the association of the heterochromatin with the inner nuclear membrane. Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins.

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

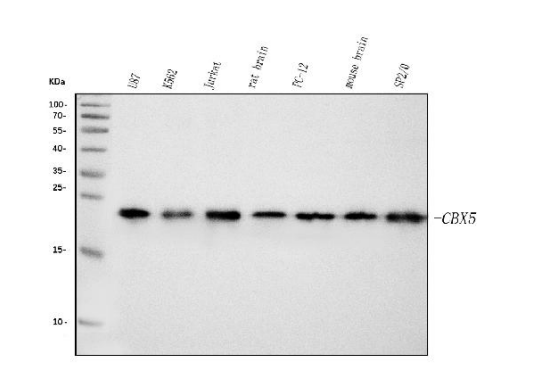

Figure 1. Western blot analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.

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.

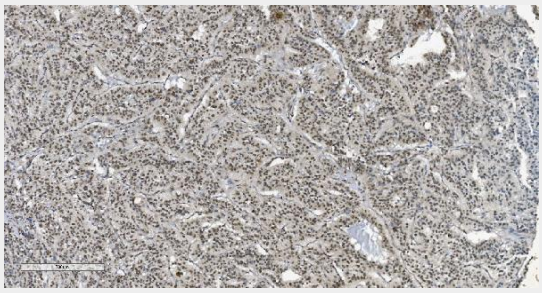

Figure 2. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human adrenocortical adenoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section 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.

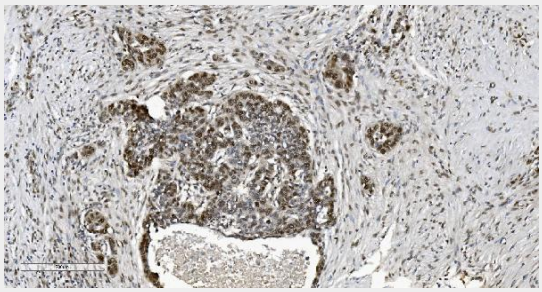

Figure 3. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue 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.

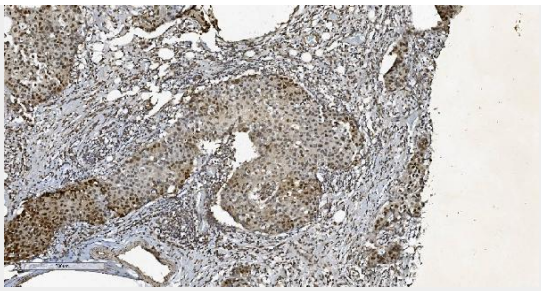

Figure 4. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked 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.

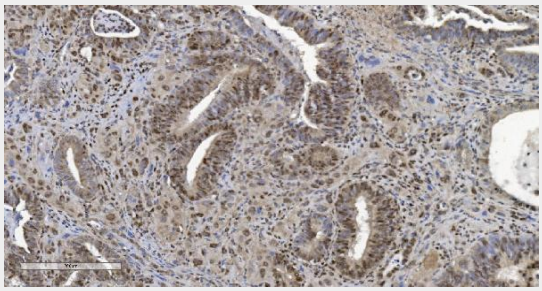

Figure 5. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section 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.

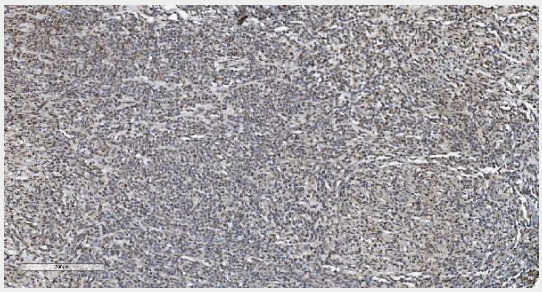

Figure 6. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human lymphoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 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.

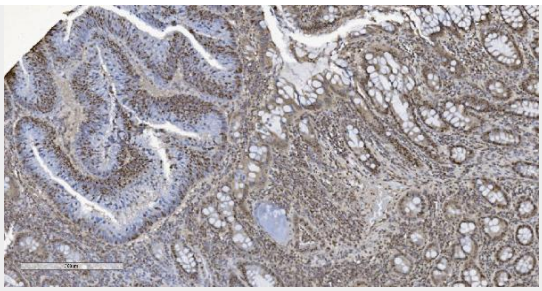

Figure 7. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked 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.

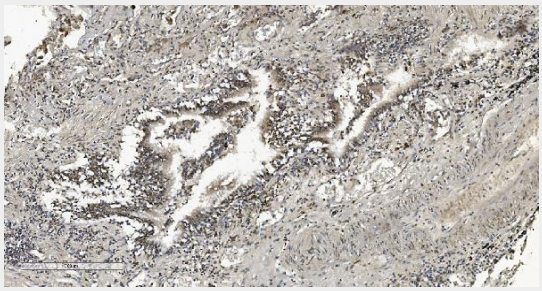

Figure 8. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section 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.

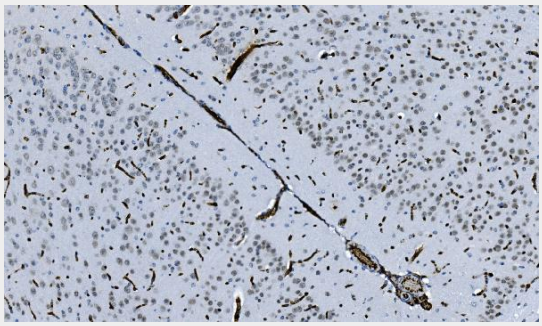

Figure 9. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 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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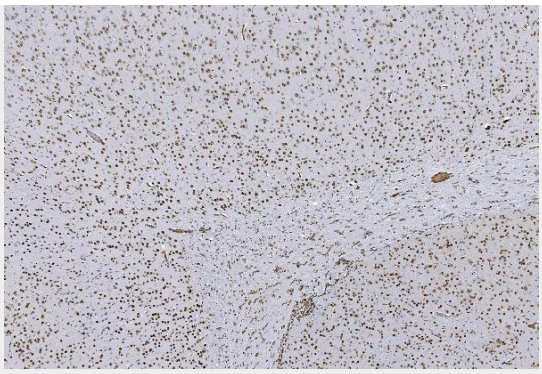

Figure 10. IHC analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 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.

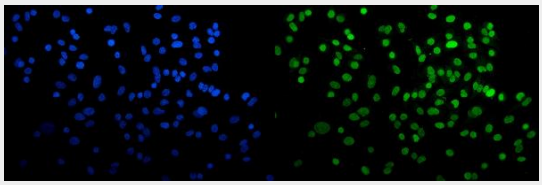

Figure 11. IF analysis of HP1 alpha/CBX5 using anti-HP1 alpha/CBX5 antibody (M02780-2).
HP1 alpha/CBX5 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat 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.

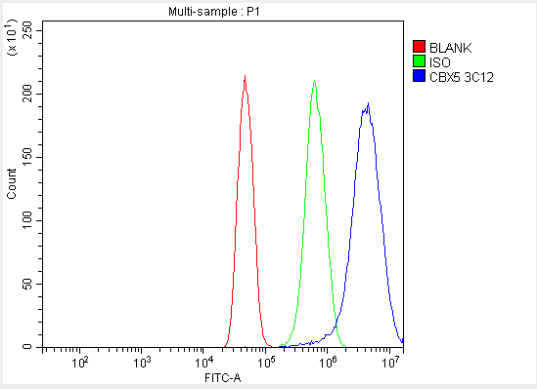

Figure 12. Flow Cytometry analysis of U251 cells using anti-HP1 alpha/CBX5 antibody (M02780-2).
Overlay histogram showing U251 cells stained with M02780-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HP1 alpha/CBX5 Antibody (M02780-2, 1 µg/1x10^6 cells) 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.