abcam

Product datasheet

Anti-HP1 gamma/CBX3 (phospho S93) antibody ab45270

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Overview

Product name Anti-HP1 gamma/CBX3 (phospho S93) antibody

Description Rabbit polyclonal to HP1 gamma/CBX3 (phospho S93)

Host species Rabbit

Tested applications Suitable for: ICC/IF, IP, WB, IHC-P

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Cow, Rhesus monkey, Orangutan

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

1

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab45270 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use a concentration of 5 µg/ml.
IP		Use a concentration of 5 µg/ml.
WB	★★★★ (2)	Use a concentration of 1 µg/ml. Detects a band of approximately 24 kDa (predicted molecular weight: 21 kDa).
IHC-P	★★★★☆ (1)	Use a concentration of 1 µg/ml.

Target

Function

Seems to be involved in transcriptional silencing in heterochromatin-like complexes. Recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression. May contribute to the association of the heterochromatin with the inner nuclear membrane through its interaction with lamin B receptor (LBR). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins.

Sequence similarities

Contains 2 chromo domains.

Post-translational modifications

Phosphorylated by PIM1. Phosphorylated during interphase and possibly hyper-phosphorylated

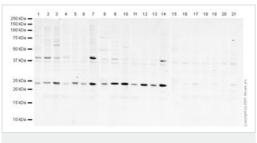
difications during mitosis.

Cellular localization

Nucleus. Associates with euchromatin and is largely excluded from constitutive heterochromatin.

 $\label{eq:may-be-associated} \mbox{ May be associated with microtubules and mitotic poles during mitosis.}$

Images



Western blot - Anti-HP1 gamma/CBX3 (phospho S93) antibody (ab45270) **All lanes :** Anti-HP1 gamma/CBX3 (phospho S93) antibody (ab45270) at 1 μg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell

Lysate

Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell

Lysate

Lane 3: A431 (Human epithelial carcinoma cell line) Whole Cell

Lysate

Lane 4: HEK293 Human embryonic kidney cell line Whole Cell

Lysate

Lane 5: HepG2 (Human hepatocellular liver carcinoma cell line)

Whole Cell Lysate

Lane 6 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lane 7 : SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate

Lane 8 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 peptide ($\underline{ab51840}$) at 1 $\mu g/ml$

Lane 9 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 peptide ($\underline{ab51840}$) at 1 $\mu g/ml$

Lane 10 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 peptide ($\underline{ab51840}$) at 1 $\mu g/ml$

Lane 11 : HEK293 Human embryonic kidney cell line Whole Cell Lysate with Human HP1 gamma/CBX3 peptide (ab51840) at 1 μ g/ml

Lane 12 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 peptide (ab51840) at 1 μ g/ml

Lane 13 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate ($\underline{ab28419}$) with Human HP1 gamma/CBX3 peptide ($\underline{ab51840}$) at 1 μ g/ml

Lane 14 : SH-SY5Y (Human neuroblastoma cell line) Whole Cell Lysate ($\underline{ab45968}$) with Human HP1 gamma/CBX3 peptide ($\underline{ab51840}$) at 1 μ g/ml

Lane 15: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide (ab45386) at 1 µg/ml

Lane 16: Jurkat (Human T cell lymphoblast-like cell line) Whole
Cell Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide
(ab45386) at 1 µg/ml

Lane 17: A431 (Human epithelial carcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide (**ab45386**) at 1 μg/ml

Lane 18: HEK293 Human embryonic kidney cell line Whole Cell Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide (ab45386) at 1 µg/ml

Lane 19 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide (ab45386) at 1 μ g/ml

Lane 20 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide ($\underline{ab45386}$) at 1 μ g/ml

Lane 21: SHSY-5Y (Human neuroblastoma cell line) Whole Cell

Lysate with Human HP1 gamma/CBX3 (phospho S93) peptide ($\underline{ab45386}$) at 1 μ g/ml

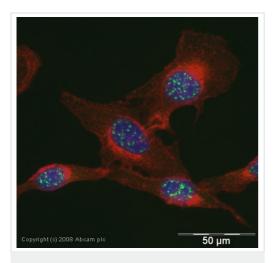
Lysates/proteins at 10 µg per lane.

Secondary

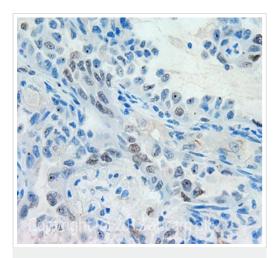
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit lgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 21 kDa



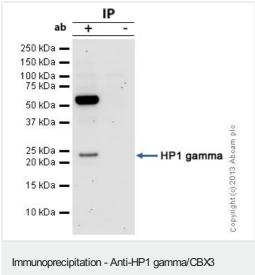
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 (phospho S93) antibody (ab45270) ICC/IF image of ab45270 stained human HeLa cells. The cells were methanol fixed (5 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab45270, 5µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 gamma/CBX3 (phospho S93) antibody (ab45270)

IHC image of HP1 gamma/CBX (phospho S83) staining in Human lung adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab45270, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



(phospho S93) antibody (ab45270)

HP1 gamma/CBX was immunoprecipitated using 0.5mg HepG2 whole cell extract, 5µg of Rabbit polyclonal to HP1 gamma/CBX and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, HepG2 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab45270.

Secondary: Clean-Blot IP Detection Reagent (HRP) at 1/500 dilution.

Band: 22kDa; HP1 gamma/CBX

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