abcam

Product datasheet

Anti-Histone H2A.X antibody - ChIP Grade ab47503

★★★★★ 1 Abreviews 1 References 画像数 5

製品の概要

製品名	Anti-Histone H2A.X antibody - ChIP Grade
製品の詳細	Rabbit polyclonal to Histone H2A.X - ChIP Grade
由来種	Rabbit
特異性	This antibody detects endogenous levels of total Histone H2A.X protein. ab47503 reacts with non-phosphorylated form of Histone H2A.X and not the phosphorylated form of the protein.
アプリケーション	**適用あり:** ChIP, ICC/IF, ELISA, WB, IHC-P
種交差性	**交差種:** Human **交差が予測される動物種:** Mouse
免疫原	Synthetic peptide corresponding to Human Histone H2A.X aa 138-140 (phospho S140). A sinthetic non-phosphopeptide derived from human Histone H2A.X around the phosphorylation site of serine 140 (Q-A-SP-Q-E) Sequence: (Q-A-SP-Q-E) Database link: **P16104** Run BLAST with Run BLAST with
ポジティブ・コントロール	Hela cell extracts; Human breast carcinoma tissue. IHC-P: Human normal colon FFPE tissue sections. ICC/IF: HeLa cells.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	Preservative: 0.02% Sodium Azide Constituents: 50% Glycerol, PBS, 150mM Sodium chloride, pH 7.4
精製度	Immunogen affinity purified
特記事項(精製)	The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.

ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab47503の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ChIP		Use 2 µg for 25 µg of chromatin.
ICC/IF		Use a concentration of 1 µg/ml.
ELISA		1/10000.
WB	★★★★★ (1)	1/500 - 1/1000. Detects a band of approximately 42 kDa (predicted molecular weight: 15 kDa).
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ターゲット情報

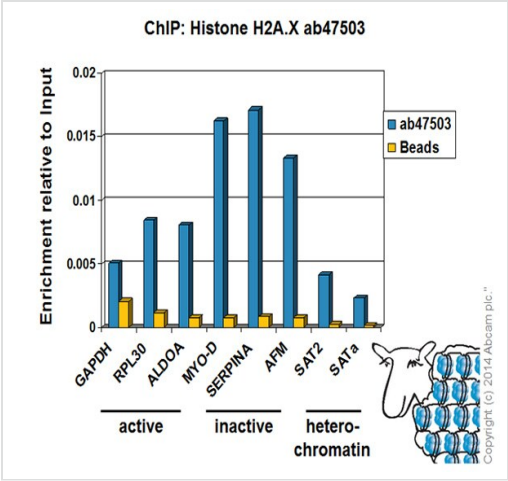
機能	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. Required for checkpoint-mediated arrest of cell cycle progression in response to low doses of ionizing radiation and for efficient repair of DNA double strand breaks (DSBs) specifically when modified by C-terminal phosphorylation.
配列類似性	Belongs to the histone H2A family.
発生段階	Synthesized in G1 as well as in S-phase.
ドメイン	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
翻訳後修飾	Phosphorylated on Ser-140 (to form gamma-H2AFX or H2AX139ph) in response to DNA double strand breaks (DSBs) generated by exogenous genotoxic agents and by stalled replication forks, and may also occur during meiotic recombination events and immunoglobulin class switching in lymphocytes. Phosphorylation can extend up to several thousand nucleosomes from the actual site of the DSB and may mark the surrounding chromatin for recruitment of proteins required for DNA damage signaling and repair. Widespread phosphorylation may also serve to amplify the damage signal or aid repair of persistent lesions. Phosphorylation of Ser-140 (H2AX139ph) in response to ionizing radiation is mediated by both ATM and PRKDC while defects in DNA replication induce Ser-140 phosphorylation (H2AX139ph) subsequent to activation of ATR and PRKDC. Dephosphorylation of Ser-140 by PP2A is required for DNA DSB repair. In meiosis, Ser-140 phosphorylation (H2AX139ph) may occur at synaptonemal complexes during leptotene as an ATM-dependent response to the formation of programmed DSBs by SPO11. Ser-140

phosphorylation (H2AX139ph) may subsequently occurs at unsynapsed regions of both autosomes and the XY bivalent during zygotene, downstream of ATR and BRCA1 activation. Ser-140 phosphorylation (H2AX139ph) may also be required for transcriptional repression of unsynapsed chromatin and meiotic sex chromosome inactivation (MSCI), whereby the X and Y chromosomes condense in pachytene to form the heterochromatic XY-body. During immunoglobulin class switch recombination in lymphocytes, Ser-140 phosphorylation (H2AX139ph) may occur at sites of DNA-recombination subsequent to activation of the activation-induced cytidine deaminase AICDA. Phosphorylation at Tyr-143 (H2AXY142ph) by BAZ1B/WSTF determines the relative recruitment of either DNA repair or pro-apoptotic factors. Phosphorylation at Tyr-143 (H2AXY142ph) favors the recruitment of APBB1/FE65 and pro-apoptosis factors such as MAPK8/JNK1, triggering apoptosis. In contrast, dephosphorylation of Tyr-143 by EYA proteins (EYA1, EYA2, EYA3 or EYA4) favors the recruitment of MDC1-containing DNA repair complexes to the tail of phosphorylated Ser-140 (H2AX139ph). Monoubiquitination of Lys-120 (H2AXK119ub) by RING1 and RNF2/RING2 complex gives a specific tag for epigenetic transcriptional repression. Following DNA double-strand breaks (DSBs), it is ubiquitinated through 'Lys-63' linkage of ubiquitin moieties by the E2 ligase UBE2N and the E3 ligases RNF8 and RNF168, leading to the recruitment of repair proteins to sites of DNA damage. Monoubiquitination and ionizing radiation-induced 'Lys-63'-linked ubiquitination are distinct events.

細胞内局在

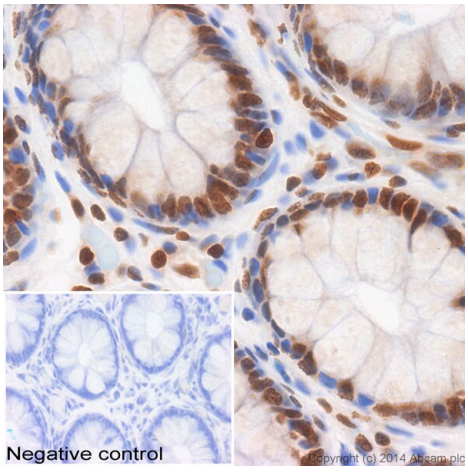
Nucleus. Chromosome.

画像



ChIP - Anti-Histone H2A.X antibody - ChIP Grade (ab47503)

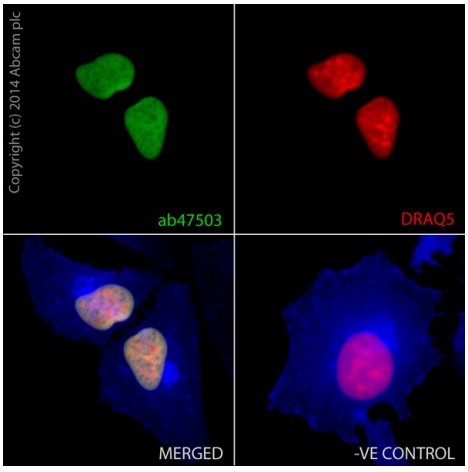
Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab47503 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody - ChIP Grade (ab47503)

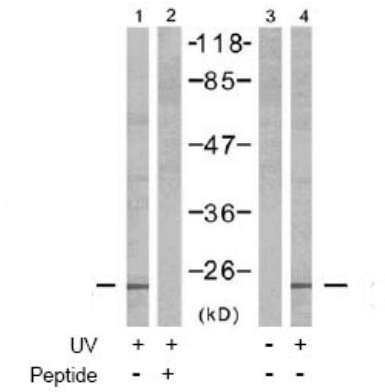
IHC image of ab47503 staining Histone H2A.X in human colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab47503, 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. No primary antibody was used in the negative control (shown on the inset).

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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody - ChIP Grade (ab47503)

ab47503 staining Histone H2A.X in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab47503 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 2 µg/ml (shown in green). AlexaFluor®350 WGA was used at a 1/200 dilution and incubated for 1h with the cells, to label plasma membranes (shown in blue). Nuclear DNA was labelled in red with 1.25 µM DRAQ5™ (**ab108410**), which was added to the secondary antibody mixture. A secondary only negative control is displayed, which indicates that the Histone H2A.X staining observed is due to primary antibody specificity and not to unspecific binding of the secondary antibody to the cells.

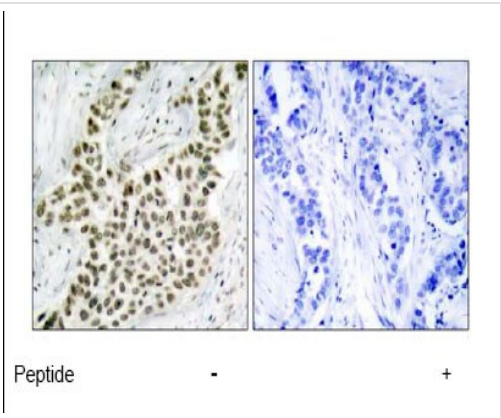


Western blot - Anti-Histone H2A.X antibody - ChIP Grade (ab47503)

Lanes 1-2 : Anti-Histone H2A.X antibody - ChIP Grade (ab47503) at 1/500 dilution
Lanes 3-4 : Anti-Histone H2A.X (phospho S140) antibody (**ab47371**) at 1/500 dilution

Lanes 1 & 4 : Extracts from Hela cells treated with UV
Lane 2 : Extracts from Hela cells treated with UV with blocking peptide
Lane 3 : Extracts from Hela cells

Predicted band size: 15 kDa
Observed band size: 15 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody - ChIP Grade (ab47503)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using ab47503 at a 1/50 dilution.
Left image : Untreated

Right image : Treated with Peptide

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.co.jp/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors