abcam

Product datasheet

Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free ab173324

リコンビナント RabMAb

画像数 16

製品の概要

製品名	Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free
製品の詳細	Rabbit monoclonal [Y47] to Histone H3 (di methyl K4) - BSA and Azide free
由来種	Rabbit
特異性	This antibody only detects Histone H3 dimethylated on Lysine 4.
アプリケーション	**適用あり:** ChIC/CUT&RUN-seq, Flow Cyt (Intra), ChIP-sequencing, ICC/IF, IP, ChIP, IHC-P, WB
種交差性	**交差種:** Mouse, Rat, Chicken, Cow, Human, African green monkey **交差が予測される動物種:** Monkey, Rice
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HeLa, HEK-293, SH-SY5Y, C6 L6, NIH/3T3, COS-1, UMNSAH/DF-1 and MDBK cell lysates. ICC/IF: HepG2 cells. Flow Cyt (intra): HeLa and HepG2 cells. IHC-P: Human cervical carcinoma, mouse colon and rat spleen tissues. ChIP: Chromatin prepared from Hela cells, ALDO ChIP primer pair **ab269260**. ChIP-seq: Chromatin prepared from Hela cells. IP: Hep-G2 cells. ChIC/CUT&RUN-seq: HeLa cells.
特記事項	ab173324 is the carrier-free version of **ab32356**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity

- Long-term security of supply
- Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y47
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ab173324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。

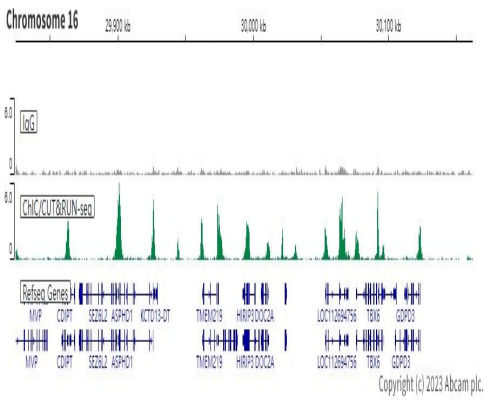
アプリケーション	Abreviews	特記事項
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).

ターゲット情報

機能	Core component of nucleosome. 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.
配列類似性	Belongs to the histone H3 family.
発生段階	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
翻訳後修飾	Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me). Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription. Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression. Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters. Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin. Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun. Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C. Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate

	DNA accessibility to repair proteins.
細胞内局在	Nucleus. Chromosome.

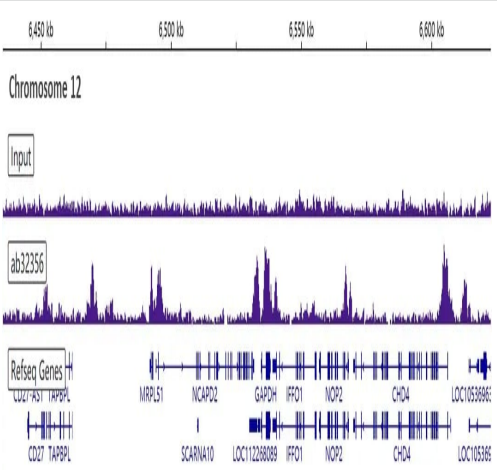
画像



ChIC/CUT&RUN sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/µL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5 µg of **ab32356** [Y47]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.
Additional screenshots of mapped reads can be downloaded **here**.
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

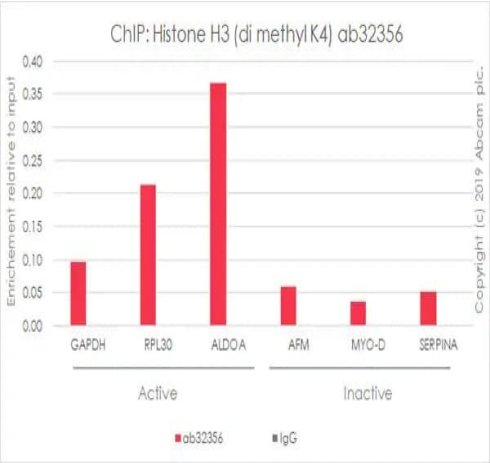


ChIP-sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 4 µg of Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (**ab32356**). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

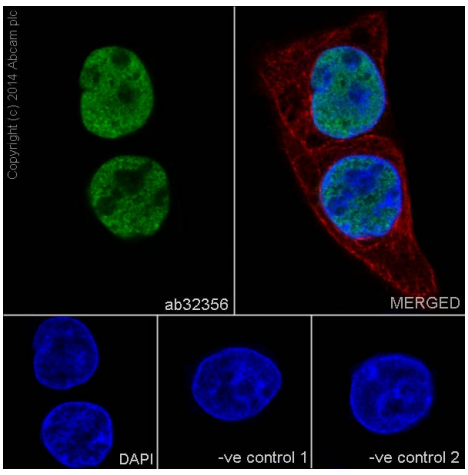
Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

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 **ab32356** (red), and 20µl of Anti Rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.



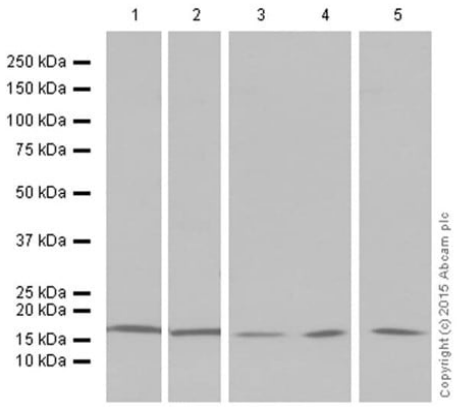
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Histone H3 (di methyl K4) with purified **ab32356** at a dilution of 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with **ab7291**, a mouse anti-tubulin (1/1000) using **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).

Control 1: primary antibody (1/1000) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).



Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

All lanes : Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (**ab32356**) at 1/2000 dilution

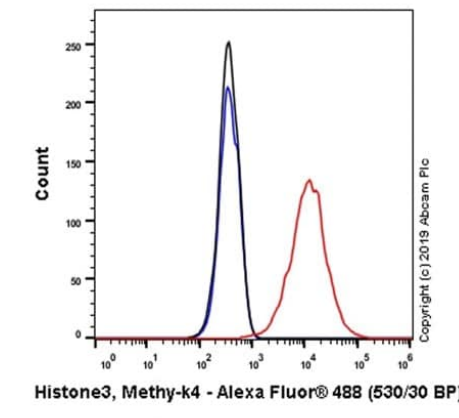
Lane 1 : L6 (Rat skeletal muscle cell line) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 3 : COS-1 (African green monkey kidney fibroblast-like cell line) whole cell lysate
Lane 4 : UMNSAH/DF-1 (Transformed chicken embryonic fibroblast cell line) whole cell lysate
Lane 5 : MDBK (Bovine kidney cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 15 kDa
Observed band size: 17 kDa

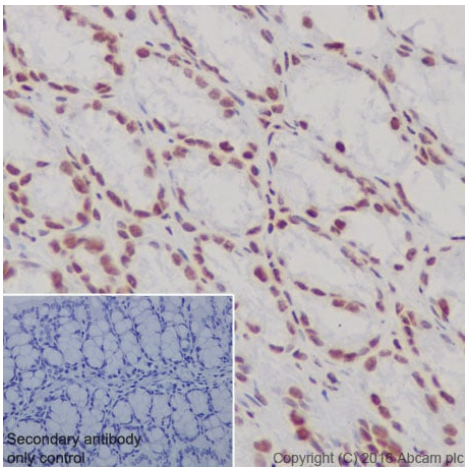
This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).



Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

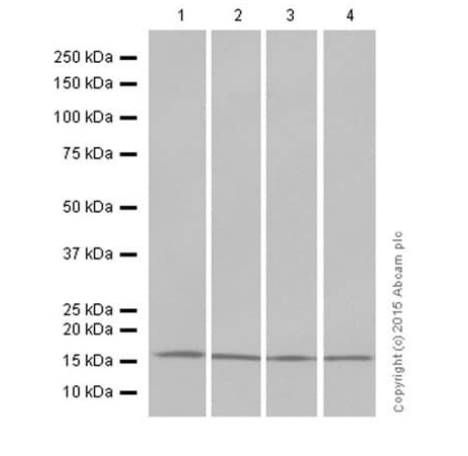
Intracellular Flow Cytometry analysis of HepG2 (human hepatocellular carcinomal) cells labeling Histone H3 di methyl K4 with purified **ab32356** at 1/500 dilution (1.17µg/ml) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) / Black. Unlabeled control - Unlabelled cells / blue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse colon tissue labelling Histone H3 (di methyl K4) with purified **ab32356** at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).



Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

All lanes : Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (**ab32356**) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate
Lane 4 : C6 (Rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 15 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

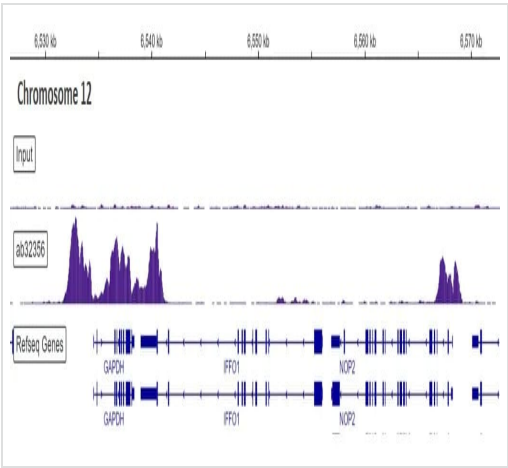
Immunoprecipitation - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

ab32356 (purified) at 1/30 dilution (20 µg/ml) immunoprecipitating Histone H3 di methyl K4 in HepG2 whole cell lysate.

Lane 1 (input): HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): **ab32356** & HepG2 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32356** in HepG2 whole cell lysate
For western blotting, **ab32356** at 1/500 and veriBlot for IP secondary antibody (HRP) (**ab131366**) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM /TBST.

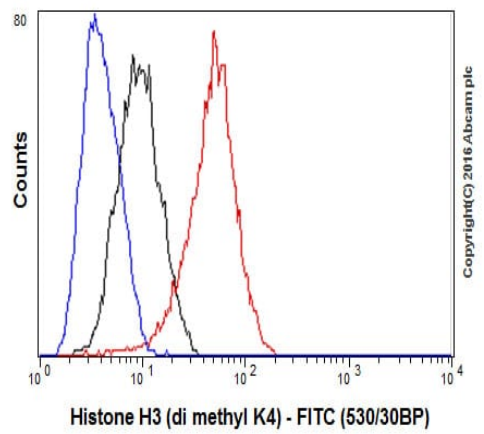


ChIP-sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

This data was developed using the same antibody clone in a different buffer formulation (**ab32356**).

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (**ab32356**). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.

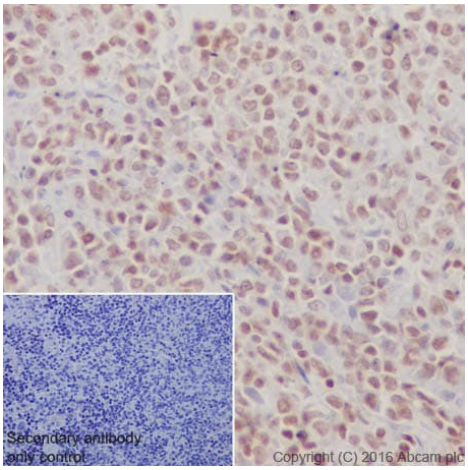
Additional screenshots of mapped reads can be downloaded **here**.



Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

Intracellular Flow Cytometry analysis of HeLa cells labelling Histone H3 (di methyl K4) with purified **ab32356** at 1/150 (red). Cells were fixed with 80% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (**ab172730**). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

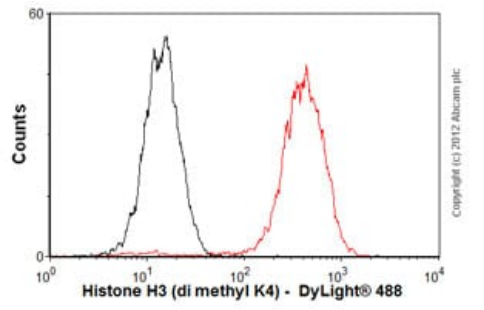
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue labelling Histone H3 (di methyl K4) with purified **ab32356** at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).

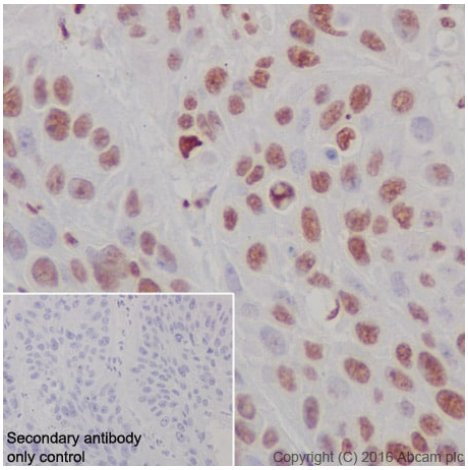


Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

Overlay histogram showing HeLa cells stained with unpurified **ab32356** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab32356**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight® 488 (IgG; H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a

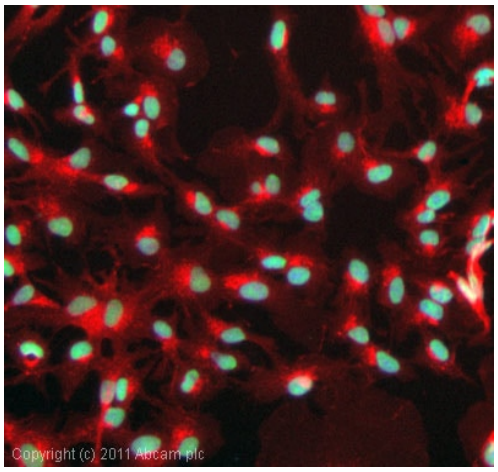
different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling Histone H3 (di methyl K4) with purified **ab32356** at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.

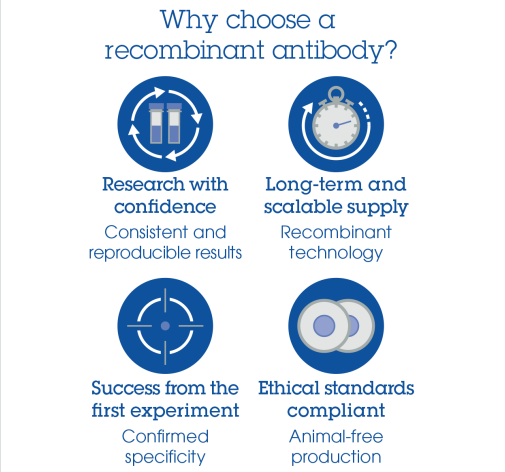
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

ICC/IF image of unpurified **ab32356** stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (**ab32356** , 5µg/ml) overnight at +4°C. The secondary antibody (green) was a goat **anti-rabbit DyLight® 488** (IgG - H&L, pre-adsorbed) (**ab96899**) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32356**).



Anti-Histone H3 (di methyl K4) antibody [Y47] - BSA and Azide free (ab173324)

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