abcam

Product datasheet

Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] ab14955

24 Abreviews 216 References 10 Images

Overview

Product name	Anti-Histone H3 (phospho S10) antibody [mAbcam 14955]
Description	Mouse monoclonal [mAbcam 14955] to Histone H3 (phospho S10)
Host species	Mouse
Specificity	ab14955 recognises phospho S10 on Histone H3. It recognises a phospho S28 peptide by ELISA, but is not blocked by phospho S28 in WB.
Tested applications	**Suitable for:** IHC-P, ELISA, Flow Cyt (Intra), ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Human, Drosophila melanogaster **Predicted to work with:** Rat, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Indian muntjac, Monkey, Schizosaccharomyces pombe, Zebrafish, Mammals, Tobacco, Chlamydomonas reinhardtii, African green monkey, Aspergillus nidulans, Neurospora crassa, Oncopeltus
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Control HeLa Histone Prep. Colcemid treated HeLa Histone prep. HeLa cell lysate treated with calyculin A. IHC-P: Human kidney tissue. ICC: HeLa and 10T1/2 cells. Flow Cyt (Intra): HeLa cells.
General notes	Hybridomas were prepared and the resulting clones were positively screened by ELISA against the immunising tri methyl K9 and phospho S10 dimodified peptide. Clones were also positively screened against both tri methyl K9 and phospho S10 peptides. Clones were negatively screened against the unmodified version of the peptides. This clone binds to the tri methyl K9 and phospho S10 dimodified peptide and to the phospho S10 peptide, but not to the tri methyl K9 peptide or to equivalent unmodified Histone H3 peptide. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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: 7.50 Preservative: 0.02% Sodium azide Constituent: PBS
Purity	IgG fraction
Clonality	Monoclonal
Clone number	mAbcam 14955
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab14955 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
IHC-P	★★★★★ (3)	Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF	★★★★★ (6)	Use a concentration of 1 µg/ml.
WB	★★★★☆ (7)	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).

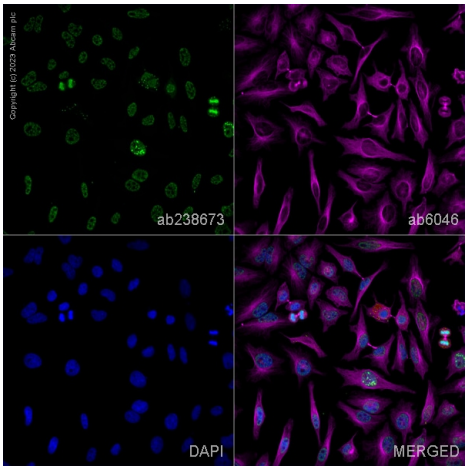
Target

Function	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.
Sequence similarities	Belongs to the histone H3 family.
Developmental stage	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
Post-translational modifications	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.

Cellular localization Nucleus. Chromosome.

Images



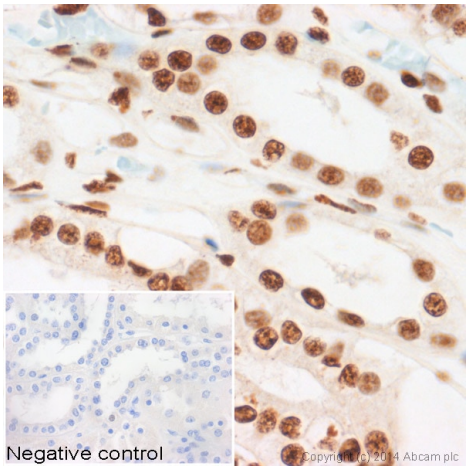
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

This data was developed using the same antibody clone in a different buffer formulation containing PBS only (**ab238673**).

ab238673 staining Histone H3 (phospho S10) in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with **ab238673** at 5µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

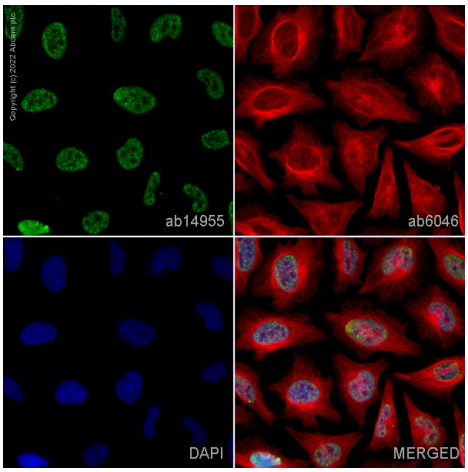


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

IHC image of ab14955 staining Histone H3 (phospho S10) in human kidney 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 (pH 6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab14955, 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 hematoxylin 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.

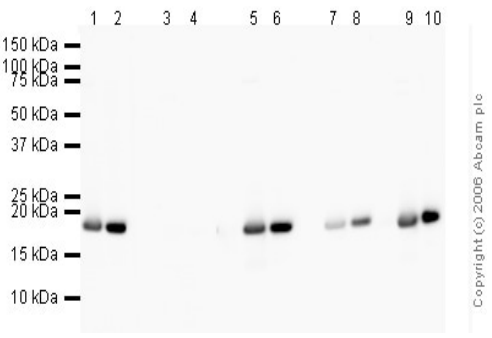


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

ab14955 staining Histone H3 (phospho S10) in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab14955 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

All lanes : Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955) at 1 µg/ml

Lane 1 : Control HeLa Histone Prep
Lane 2 : Colcemid treated HeLa Histone prep
Lane 3 : Control HeLa Histone Prep with Human Histone H3 (tri methyl K9, phospho S10) peptide (**ab15644**) at 1 µg/ml
Lane 4 : Colcemid treated HeLa Histone prep with Human Histone H3 (tri methyl K9, phospho S10) peptide (**ab15644**) at 1 µg/ml
Lane 5 : Control HeLa Histone Prep with Human Histone H3 (unmodified) peptide (**ab7228**) at 1 µg/ml
Lane 6 : Colcemid treated HeLa Histone prep with Human Histone H3 (unmodified) peptide (**ab7228**) at 1 µg/ml
Lane 7 : Control HeLa Histone Prep with Human Histone H3 (phospho S10) peptide (**ab11477**) at 1 µg/ml
Lane 8 : Colcemid treated HeLa Histone prep with Human Histone H3 (phospho S10) peptide (**ab11477**) at 1 µg/ml
Lane 9 : Control HeLa Histone Prep with Human Histone H3 (phospho S28) peptide (**ab14793**) at 1 µg/ml
Lane 10 : Colcemid treated HeLa Histone prep with Human Histone H3 (phospho S28) peptide (**ab14793**) at 1 µg/ml

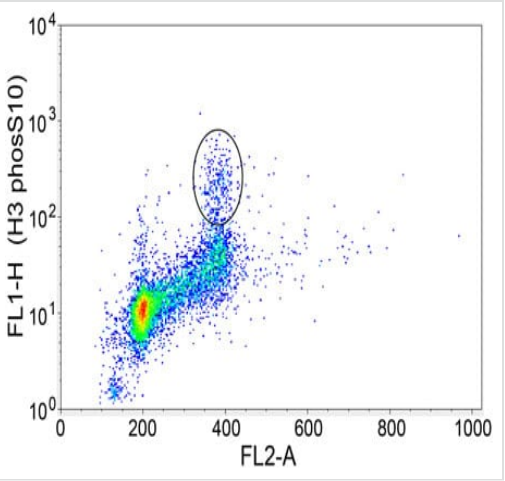
Lysates/proteins at 0.5 µg per lane.

Secondary

All lanes : Rabbit polyclonal to Mouse IgG H&L (HRP) at 1/5000 dilution

Performed under reducing conditions.

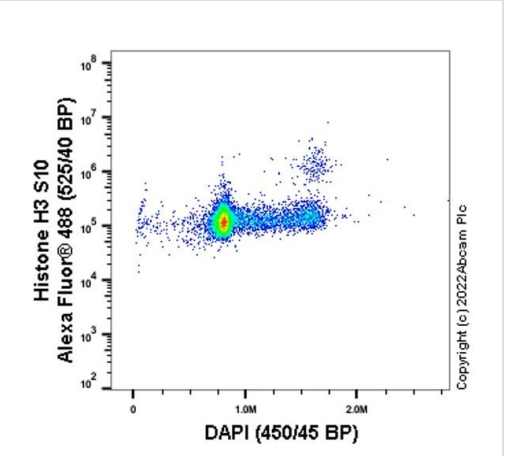
Predicted band size: 15 kDa
Observed band size: 17 kDa



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

This image is courtesy of an Abreview submitted by Dr Kirk McManus

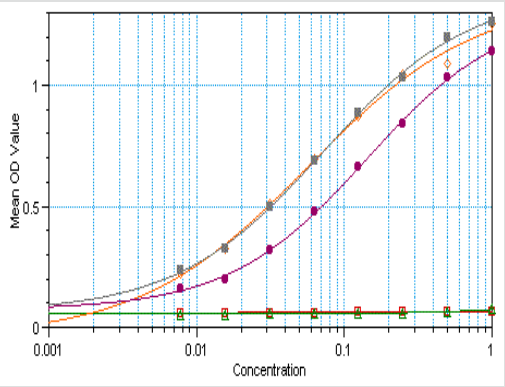
ab14955 (1/1000) stainning a population of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells positive for Histone H3 (phospho S10). Cells were trypsizined, pelleted and fixed in ice cold ethanol. Cellular debris was eliminated and the FL2-A/FL2-W was used to eliminate clumping cells. For further experimental details please refer to abreview.



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

Flow cytometry dot plot showing HeLa cells stained with ab14955 (red line). The cells were fixed with 100% ethanol. The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab14955) (1x 10^6 in 100µl at 1µg/ml (1/500)) for 30 min at 22°C. The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/4000 for 30 min at 22°C. DNA staining was performed by pre-incubation with RNase A (250µg/ml) followed by DAPI.

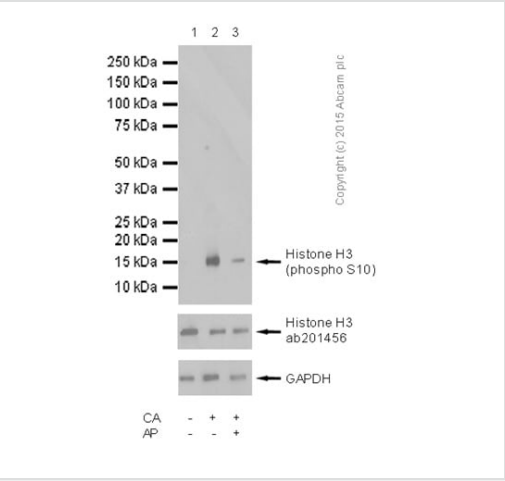
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



ELISA - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

By ELISA, ab14955 detects:
the singly modified phospho S10 peptide and the dual modified phospho S10 and tri methyl K9 peptide (the orange and grey lines respectively),
less strongly detects the phospho S28 peptide (purple line),
does not detect the equivalent non-modified Histone H3 peptide for S10 or the singly modified tri methyl K9 peptide (the 2 lines at the bottom of the figure).

The antibody recognises phospho S28 by ELISA (although a phospho S28 peptide does not block the antibody in Western blotting).



Western blot - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

All lanes : Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955) at 1/2000 dilution

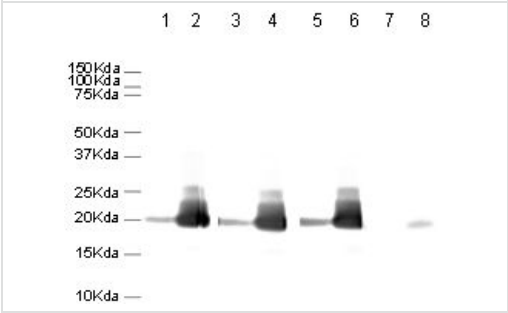
Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : HeLa cell lysate treated with calyculin A
Lane 3 : HeLa cell lysate treated with calyculin A and alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/20000 dilution

Predicted band size: 15 kDa

Exposure time: 1 second



Western blot - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

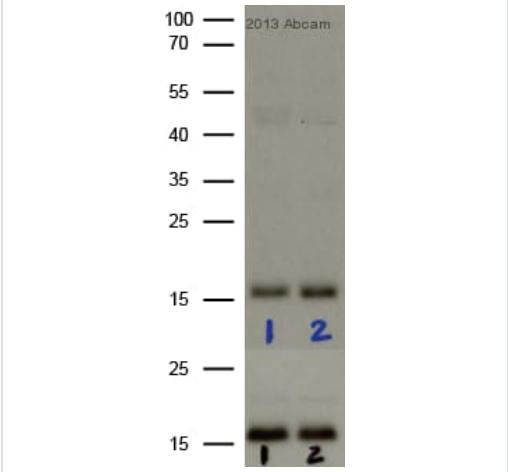
All lanes : Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955) at 0.5 µg/ml

Lane 1 : Control HeLa Histone prep
Lane 2 : Colecemid treated HeLa Histone prep
Lane 3 : Control HeLa Histone prep with Human Histone H3 (unmodified) peptide (**ab7228**) at 1 µg/ml
Lane 4 : Colecemid treated HeLa Histone prep with Human Histone H3 (unmodified) peptide (**ab7228**) at 1 µg/ml
Lane 5 : Control HeLa Histone prep with Human Histone H3 (phospho S28) peptide (**ab14793**) at 1 µg/ml
Lane 6 : Colecemid treated HeLa Histone prep with Human Histone H3 (phospho S28) peptide (**ab14793**) at 1 µg/ml
Lane 7 : Control HeLa Histone prep with Human Histone H3 (phospho S10) peptide (**ab11477**) at 1 µg/ml
Lane 8 : Colecemid treated HeLa Histone prep with Human Histone H3 (phospho S10) peptide (**ab11477**) at 1 µg/ml

Lysates/proteins at 5 µg per lane.

Secondary
All lanes : Rabbit Anti-Mouse IgG H&L (HRP) (**ab6728**) at 1/5000 dilution

Predicted band size: 15 kDa
Observed band size: 20 kDa



Western blot - Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955)

Image courtesy of Richelle Sopko, Harvard University, U.S.A

All lanes : Anti-Histone H3 (phospho S10) antibody [mAbcam 14955] (ab14955) at 1/1000 dilution

Lane 1 : Wild type 0-4 hour old fruit fly embryo lysate
Lane 2 : 0-4 hour old fruit fly embryo lysate expressing wee RNAi

Secondary
All lanes : Anti-mouse IgG, peroxidase-linked at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 5 seconds

Blocking: 10% BSA

wee shRNA embryos (lane 2) should display elevated phospho H3Ser10 levels relative to wild type

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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.com/abpromise** or contact our technical team.

Terms and conditions

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