abcam

Product datasheet

Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free ab255866

Recombinant RabMAb

10 Images

Overview

Product name	Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free
Description	Rabbit monoclonal [EPR18855-87] to RNA polymerase II CTD repeat YSPTSPS (phospho S2) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, Dot blot, IHC-P, ICC/IF, Flow Cyt, ChIP, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human RNA polymerase II CTD repeat YSPTSPS aa 1550-1650 (phospho S2). The exact sequence is proprietary. Database link: P24928
Positive control	WB: HeLa, RAW 264.7 and PC-12 whole cell lysates. Dot blot: RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide. IHC-P: Mouse testis tissue; rat testis tissue; human testis tissue. ICC/IF: HeLa and RAW 264.7 cells. Flow cyt: HeLa cells. IP: HeLa whole cell lysate. ChIP: Chromatin prepared from HeLa cells.
General notes	Ab255866 is the carrier-free version of ab238146. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. 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. ab255866 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *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.

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18855-87
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab255866** 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
WB		Use at an assay dependent concentration. Detects a band of approximately 270 kDa (predicted molecular weight: 217 kDa).

Application	Abreviews	Notes
Dot blot		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.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Function

DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Largest and catalytic component of RNA polymerase II which synthesizes mRNA precursors and many functional non-coding RNAs. Forms the polymerase active center together with the second largest subunit. Pol II is the central component of the basal RNA polymerase II transcription machinery. It is composed of mobile elements that move relative to each other. RPB1 is part of the core element with the central large cleft, the clamp element that moves to open and close the cleft and the jaws that are thought to grab the incoming DNA template. At the start of transcription, a single-stranded DNA template strand of the promoter is positioned within the central active site cleft of Pol II. A bridging helix emanates from RPB1 and crosses the cleft near the catalytic site and is thought to promote translocation of Pol II by acting as a ratchet that moves the RNA-DNA hybrid through the active site by switching from straight to bent conformations at each step of nucleotide addition. During transcription elongation, Pol II moves on the template as the transcript elongates. Elongation is influenced by the phosphorylation status of the C-terminal domain (CTD) of Pol II largest subunit (RPB1), which serves as a platform for assembly of factors that regulate transcription initiation, elongation, termination and mRNA processing. Acts as an RNA-dependent RNA polymerase when associated with small delta antigen of Hepatitis delta virus, acting both as a replicate and transcriptase for the viral RNA circular genome.

Sequence similarities

Belongs to the RNA polymerase beta' chain family.

Domain

The C-terminal domain (CTD) serves as a platform for assembly of factors that regulate transcription initiation, elongation, termination and mRNA processing.

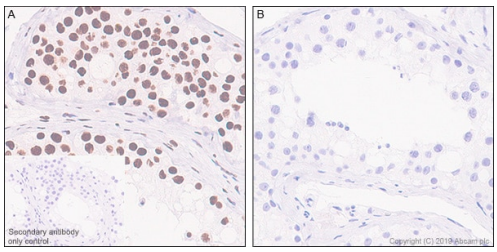
Post-translational modifications

The tandem heptapeptide repeats in the C-terminal domain (CTD) can be highly phosphorylated. The phosphorylation activates Pol II. Phosphorylation occurs mainly at residues 'Ser-2' and 'Ser-5' of the heptapeptide repeat and is mediated, at least, by CDK7 and CDK9. CDK7 phosphorylation of POLR2A associated with DNA promotes transcription initiation by triggering dissociation from DNA. Phosphorylation also takes place at 'Ser-7' of the heptapeptide repeat, which is required for efficient transcription of snRNA genes and processing of the transcripts. The phosphorylation state is believed to result from the balanced action of site-specific CTD kinases and phosphatases, and a 'CTD code' that specifies the position of Pol II within the transcription cycle has been proposed. Dephosphorylated by the protein phosphatase CTDSP1.
Among tandem heptapeptide repeats of the C-terminal domain (CTD) some do not match the Y-S-P-T-S-P-S consensus, the seventh serine residue 'Ser-7' being replaced by a lysine. 'Lys-7' in

these non-consensus heptapeptide repeats can be alternatively acetylated, methylated and dimethylated. EP300 is one of the enzyme able to acetylate 'Lys-7'. Acetylation at 'Lys-7' of non-consensus heptapeptide repeats is associated with 'Ser-2' phosphorylation and active transcription. It may regulate initiation or early elongation steps of transcription specially for inducible genes.
Methylated at Arg-1810 prior to transcription initiation when the CTD is hypophosphorylated, phosphorylation at Ser-1805 and Ser-1808 preventing this methylation. Symmetrically or asymmetrically dimethylated at Arg-1810 by PRMT5 and CARM1 respectively. Symmetric or asymmetric dimethylation modulates interactions with CTD-binding proteins like SMN1/SMN2 and TDRD3. SMN1/SMN2 interacts preferentially with the symmetrically dimethylated form while TDRD3 interacts with the asymmetric form. Through the recruitment of SMN1/SMN2, symmetric dimethylation is required for resolving RNA-DNA hybrids created by RNA polymerase II, that form R-loop in transcription terminal regions, an important step in proper transcription termination. CTD dimethylation may also facilitate the expression of select RNAs. Among tandem heptapeptide repeats of the C-terminal domain (CTD) some do not match the Y-S-P-T-S-P-S consensus, the seventh serine residue 'Ser-7' being replaced by a lysine. 'Lys-7' in these non-consensus heptapeptide repeats can be alternatively acetylated, methylated and dimethylated. Methylation occurs in the earliest transcription stages and precedes or is concomitant to 'Ser-5' and 'Ser-7' phosphorylation.
Ubiquitinated by WWP2 leading to proteasomal degradation (By similarity). Following UV treatment, the elongating form of RNA polymerase II (RNA pol IIo) is ubiquitinated UV damage sites without leading to degradation: ubiquitination is facilitated by KIAA1530/UVSSA and promotes RNA pol IIo backtracking to allow access to the nucleotide excision repair machinery.

Cellular localization Nucleus.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/2000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human testis without alkaline phosphatase treatment (panel A). No signal was detected when treated with alkaline phosphatase (panel B). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

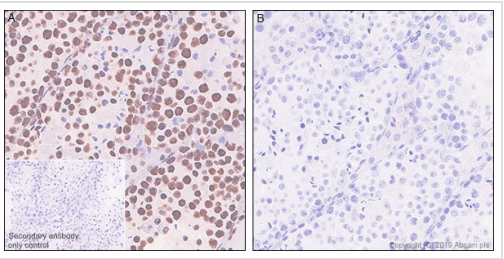
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238146 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (ab238146).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/2000 dilution, followed by a Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse testis without alkaline phosphatase treatment (panel A). No signal was detected when treated with alkaline phosphatase (panel B). Counter stained with hematoxylin.

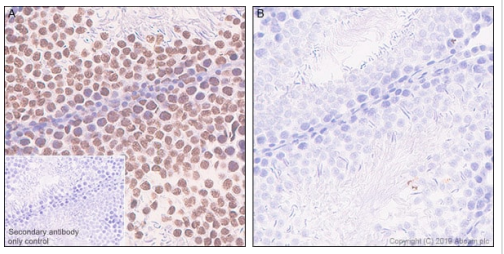
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238146 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/2000 dilution, followed by a Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on rat testis without alkaline phosphatase treatment (panel A). No signal was detected when treated with alkaline phosphatase (panel B). Counter stained with hematoxylin.

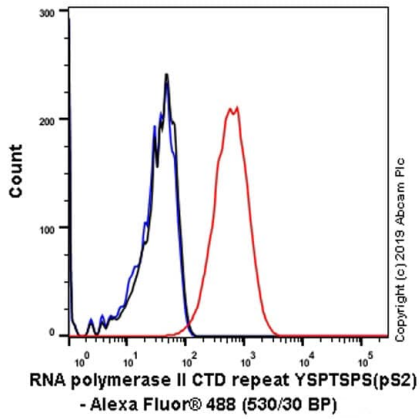
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab238146 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).

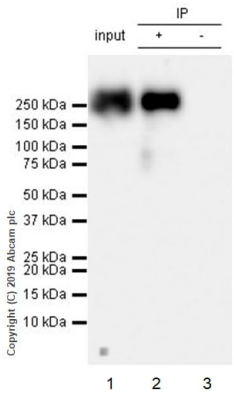


Flow Cytometry - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/600 (red) compared with Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077), at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).



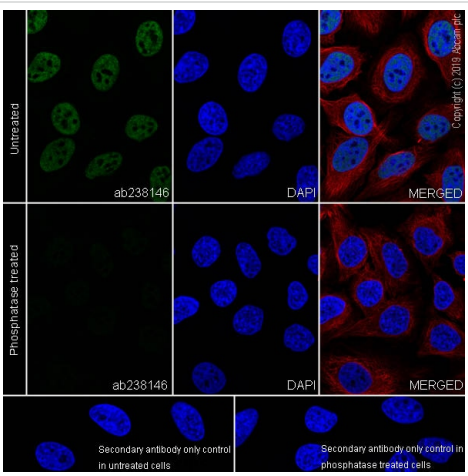
Immunoprecipitation - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

RNA polymerase II CTD repeat YSPTSPS (phospho S2) was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab238146 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab238146 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used as secondary antibody at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).
Lane 2: ab237146 IP in HeLa whole cell lysate.
Lane 3: : Rabbit monoclonal IgG (ab172730) instead of ab238146 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 7 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).

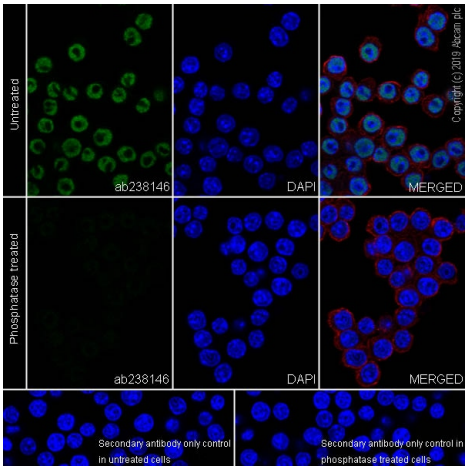


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/500 dilution, followed by a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HeLa cell line, the signal decreased after phosphatase treatment at 37°C for 2h. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).

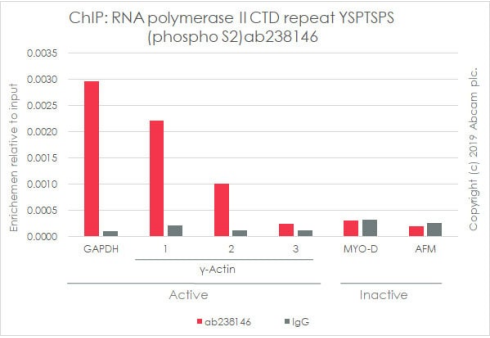


Immunocytochemistry/ Immunofluorescence - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with ab238146 at 1/500 dilution, followed by a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in RAW 264.7 cell line, the signal decreased after phosphatase treatment at 37°C for 2h. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).



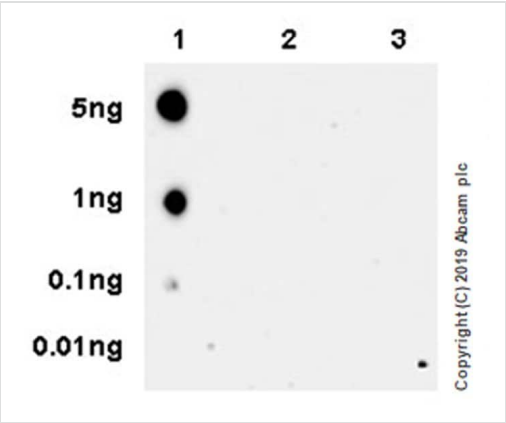
ChIP - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Chromatin was prepared from HeLa (human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab238146 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).



Dot Blot - Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

Dot blot analysis of RNA polymerase II CTD repeat YSPTSPS (phospho S2) labeled with ab238146 at 1/1000 dilution.

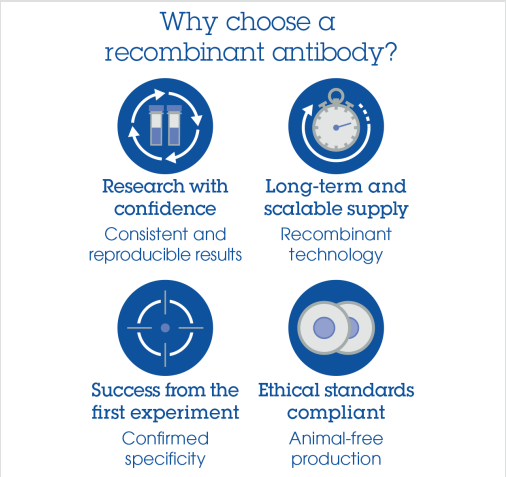
Lane 1: RNA polymerase II CTD repeat YSPTSPS (phospho S2) peptide.
Lane 2: RNA polymerase II CTD repeat YSPTSPS non-phospho peptide.
Lane 3: RNA polymerase II CTD repeat YSPTSPS (phospho S5) peptide.

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238146).



Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody [EPR18855-87] - BSA and Azide free (ab255866)

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