abcam

Product datasheet

Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free ab247375

KO VALIDATED Recombinant RabMAb

12 Images

Overview

Product name	Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free
Description	Rabbit monoclonal [EPR1568Y] to beta III Tubulin - BSA and Azide free
Host species	Rabbit
Specificity	According to BLAST analysis, it is possible that the antibody will cross-react with TBB6 (Q9BUF5), TBB2B (Q9BVA1), TBB2A (Q13885), TBB5 (P07437), TBB4A (P04350), TBB4B (P68371). This cross-reactivity has not been confirmed experimentally.
Tested applications	**Suitable for:** IHC-P, ICC/IF, WB, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SH-SY5Y, HeLa, Human cerebellum, Mouse brain and Rat brain lysate Flow Cyto (intra): HeLa cells ICC/IF: Neuro-2a cells, Hap1-TUBB3 WT/KO IHC-P: Human tonsil, Mouse and rat testis tissue sections
General notes	ab247375 is the carrier-free version of **ab68193**. 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**.

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	EPR1568Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab247375 in the following tested applications.

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

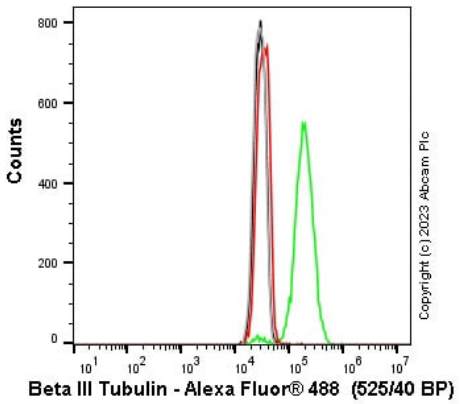
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

Function	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. TUBB3 plays a critical role in proper axon guidance and mantainance.
Tissue specificity	Expression is primarily restricted to central and peripheral nervous system.
Involvement in disease	Defects in TUBB3 are the cause of congenital fibrosis of extraocular muscles type 3A (CFEOM3A) [MIM:600638]. A congenital ocular motility disorder marked by restrictive ophthalmoplegia affecting extraocular muscles innervated by the oculomotor and/or trochlear nerves. It is clinically characterized by anchoring of the eyes in downward gaze, ptosis, and

	backward tilt of the head. Congenital fibrosis of extraocular muscles type 3 presents as a non-progressive, autosomal dominant disorder with variable expression. Patients may be bilaterally or unilaterally affected, and their oculo-motility defects range from complete ophthalmoplegia (with the eyes fixed in a hypo- and exotropic position), to mild asymptomatic restrictions of ocular movement. Ptosis, refractive error, amblyopia, and compensatory head positions are associated with the more severe forms of the disorder. In some cases the ocular phenotype is accompanied by additional features including developmental delay, corpus callosum agenesis, basal ganglia dysmorphism, facial weakness, polyneuropathy.
Sequence similarities	Belongs to the tubulin family.
Domain	The highly acidic C-terminal region may bind cations such as calcium.
Post-translational modifications	Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs exclusively on glutamate residues and results in polyglutamate chains on the gamma-carboxyl group. Also monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella) whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of such modifications is still unclear but they regulate the assembly and dynamics of axonemal microtubules.
Cellular localization	Cytoplasm > cytoskeleton.

Images



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

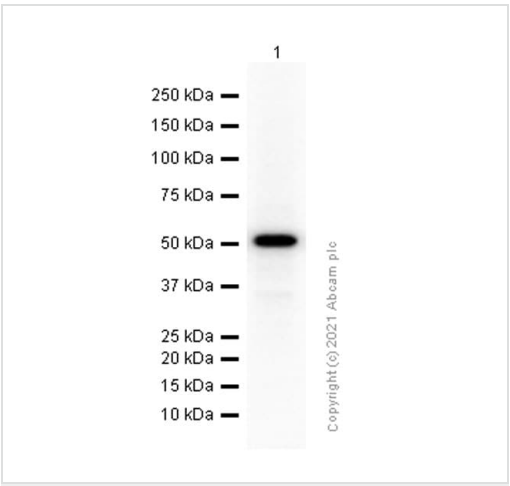
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab68193**).

Flow cytometry overlay histogram showing wild-type HeLa (green line) and TUBB3 knockout HeLa stained with **ab68193** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab68193**) (1x 10^6 in 100µl at 0.04 µg/ml (1/49250)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type HeLa - black line, TUBB3 knockout HeLa - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

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



Western blot - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

Anti-beta III Tubulin antibody [EPR1568Y] (**ab68193**) at 1/50000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

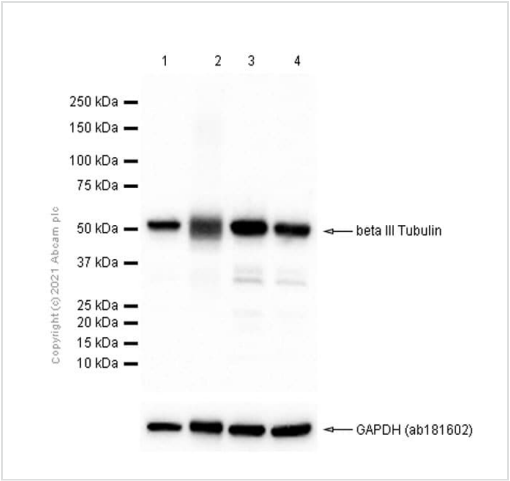
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 50 kDa

Observed band size: 50 kDa

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.



Western blot - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

All lanes : Anti-beta III Tubulin antibody [EPR1568Y] (**ab68193**) at 1/50000 dilution (Purified)

Lane 1 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate
Lane 2 : Human cerebellum lysate
Lane 3 : Mouse brain lysate
Lane 4 : Rat brain lysate

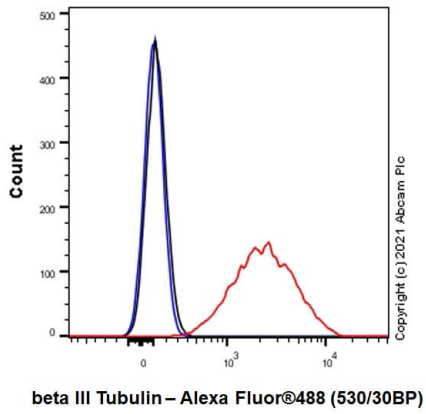
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

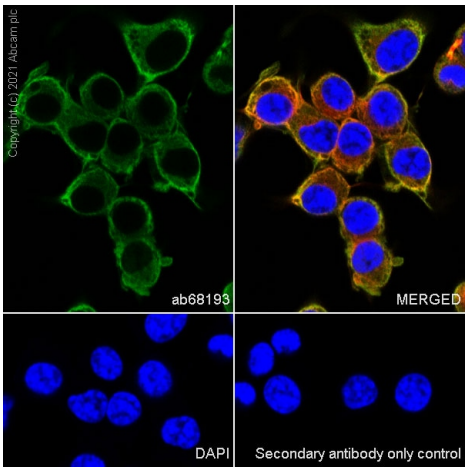
Predicted band size: 50 kDa

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.



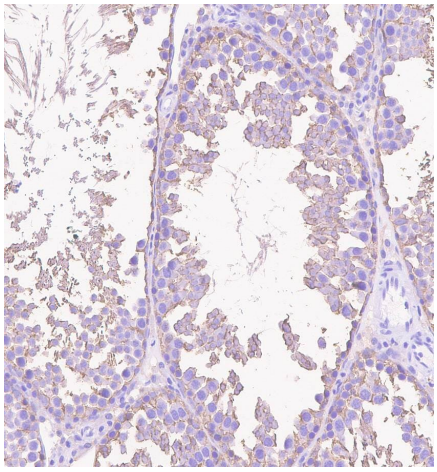
Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.
Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling beta III Tubulin with Purified **ab68193** at 1:20 dilution (10 µg/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. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



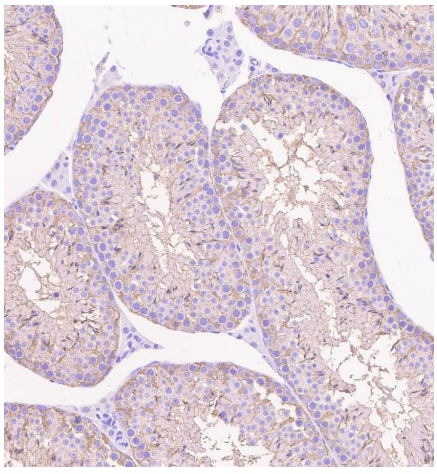
Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of Neuro-2a(Mouse neuroblastoma neuroblast) cells labeling beta III Tubulin with Purified **ab68193** at 1:50 dilution (2.2 µg/ml). Cells were fixed in 100% Methanol and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



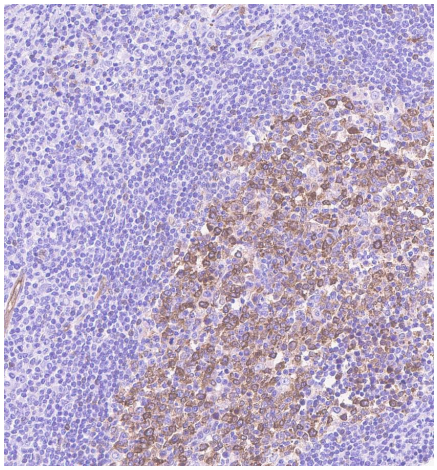
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat testis tissue sections labeling beta III Tubulin with Purified **ab68193** at 1:8000 dilution (0.014 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

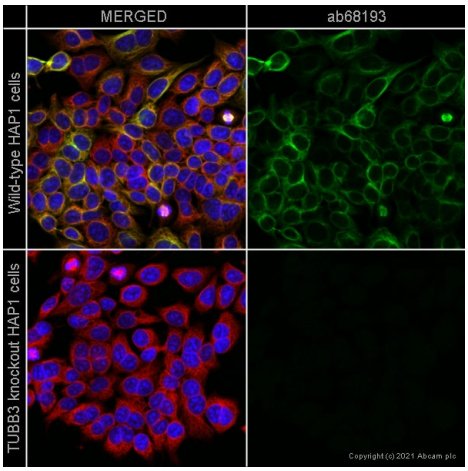
This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse testis tissue sections labeling beta III Tubulin with Purified **ab68193** at 1:8000 dilution (0.014 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling beta III Tubulin with Purified **ab68193** at 1:8000 dilution (0.014 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.

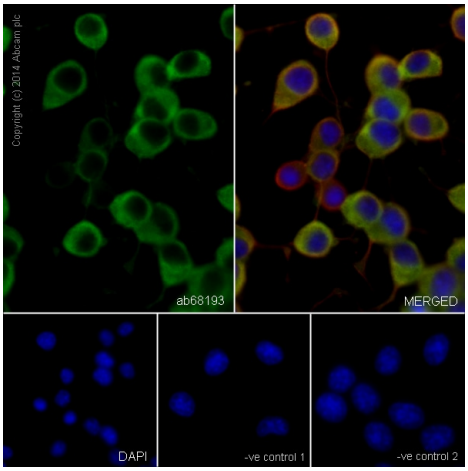


Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.

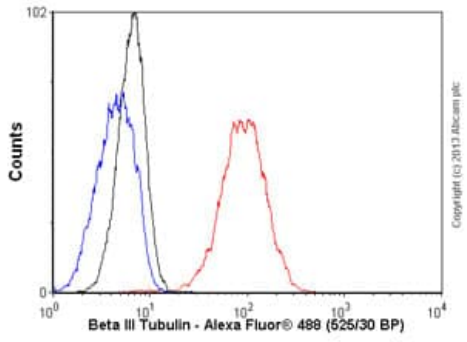
ab68193 staining beta III Tubulin in wild-type HAP1 cells (top panel) and TUBB3 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab68193** at 1µg/ml concentration and **ab7291**, a mouse anti-tubulin antibody, at 1µg/ml overnight at +4°C. This is followed by a further incubation at room temperature for 1h with a goat anti-rabbit IgG Alexa Fluor® 488 (**ab150081**) at 2µg/ml (shown in green) and a goat anti-mouse IgG Alexa Fluor® 647 (**ab150119**) at 2µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



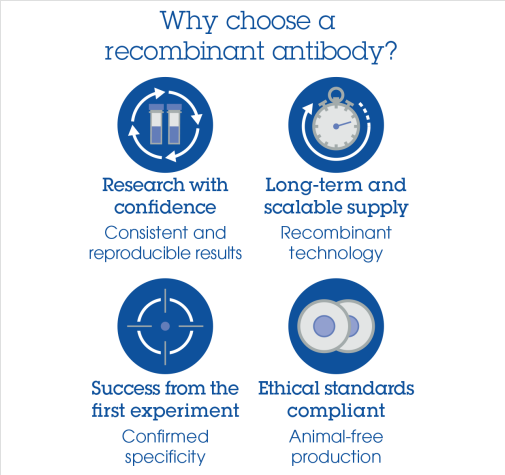
Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.**ab68193** staining beta III Tubulin in Neuro-2a 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 **ab68193** at 1µg/ml and **ab7291** 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 (**ab150081**) at 2 µg/ml (shown in green) and AlexaFluor®594 Goat anti-Mouse secondary (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.
Negative controls: 1– Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

This data was developed using **ab68193**, the same antibody clone in a different buffer formulation.Overlay histogram showing HeLa cells stained with **ab68193** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 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 (**ab68193**, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was **anti-rabbit Alexa Fluor® 488** (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 20 min used under the same conditions.



Anti-beta III Tubulin antibody [EPR1568Y] - BSA and Azide free (ab247375)

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