

Product datasheet

Anti-STAT3 (phospho Y705) antibody [EPR23968-52] ab267373

KO VALIDATED Recombinant RabMAb[®]

[24 References](#) [10 Images](#)

Overview

Product name	Anti-STAT3 (phospho Y705) antibody [EPR23968-52]
Description	Rabbit monoclonal [EPR23968-52] to STAT3 (phospho Y705)
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, IP, Flow Cyt (Intra), ChIP, Dot blot, ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa serum starved overnight, treated 30 mins 50ng/ml IFN alpha; HepG2 serum starved overnight, treated 30 mins, 100ng/ml IL-6; Jurkat treated 30 mins, 50ng/ml IFN alpha. IHC-P: Human endometrial carcinoma, tonsil tissue. ICC/IF: HeLa cells treated with IFN-alpha (50 ng/ml) for 30 min. Flow Cyt (intra): Jurkat treated (30 mins) with 50ng/ml IFN alpha. IP: Jurkat treated (30 mins) with 50ng/ml IFN alpha. Dot: STAT3 phospho Y705 peptide (aa700-710) ChIP: HepG2 (starved overnight) treated with IL-6.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR23968-52
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab267373 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		1/1000. Predicted molecular weight: 88 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.
Flow Cyt (Intra)		1/500.
ChIP		Use 5 µg for 25 µg of chromatin.
Dot blot		1/1000.
ICC/IF		1/500.

Target

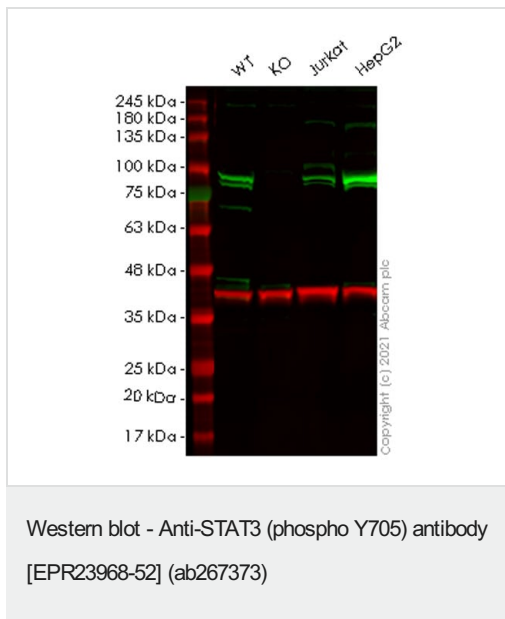
Function	Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Activated by IL31 through IL31RA. Involved in cell cycle regulation by inducing the expression of key genes for the progression from G1 to S phase, such as CCND1 (PubMed:17344214). Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role by transactivating BIRC5 expression under LEP activation (PubMed:18242580). Cytoplasmic STAT3 represses macroautophagy by inhibiting EIF2AK2/PKR activity.
Tissue specificity	Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
Involvement in disease	Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant Autoimmune disease, multisystem, infantile-onset
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through tyrosine phosphorylation by BMX. Tyrosine phosphorylated in response to IL6, IL11, LIF, CNTF,

KITLG/SCF, CSF1, EGF, PDGF, IFN-alpha, LEP and OSM. Activated KIT promotes phosphorylation on tyrosine residues and subsequent translocation to the nucleus. Phosphorylated on serine upon DNA damage, probably by ATM or ATR. Serine phosphorylation is important for the formation of stable DNA-binding STAT3 homodimers and maximal transcriptional activity. ARL2BP may participate in keeping the phosphorylated state of STAT3 within the nucleus. Upon LPS challenge, phosphorylated within the nucleus by IRAK1. Upon erythropoietin treatment, phosphorylated on Ser-727 by RPS6KA5. Phosphorylation at Tyr-705 by PTK6 or FER leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.

Cellular localization

Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Translocated into the nucleus upon tyrosine phosphorylation and dimerization, in response to signaling by activated FGFR1, FGFR2, FGFR3 or FGFR4. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. Identified in a complex with LYN and PAG1.

Images



All lanes : Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lane 2 : STAT3 knockout HeLa serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lane 3 : Jurkat (human t cell leukemia cell line from peripheral blood) treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lane 4 : HepG2 (human hepatocellular carcinoma epithelial cell) serum starved overnight, then treated with 100 ng/ml IL-6 for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/10000 dilution

Predicted band size: 88 kDa

Observed band size: 88 kDa

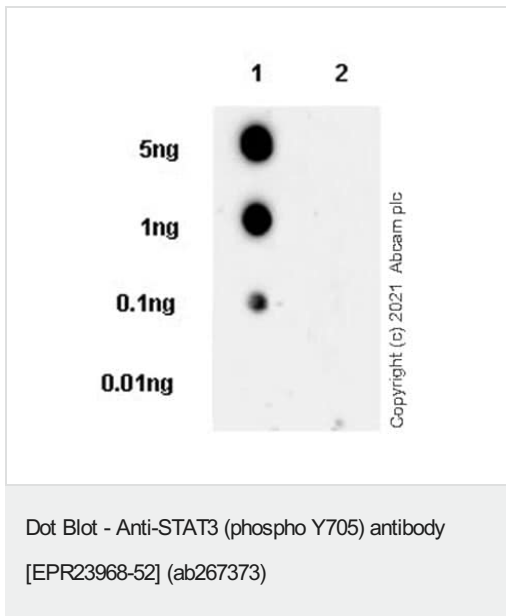
Blocking and diluting buffer and concentration: Intercept® (TBS)

Blocking Buffer diluted with an equal volume of 0.1% TBS.

Lanes 1-4: Merged signal (red and green). Green - ab267373 observed at 88 kDa. Red - loading control **ab8245** (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

Lanes 1-2: ab267373 Anti-STAT3 (phospho Y705) antibody [EPR23968-52] was shown to specifically react with STAT3 in wild-type serum starved and then IFN alpha treated HeLa cells. Loss of signal was observed when serum starved and then IFN alpha treated knockout cell line **ab255436** (knockout cell lysate **ab263797**) was used. Wild-type and STAT3 knockout samples were subjected to SDS-PAGE. ab267373 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

Lysates loaded onto lanes 3-4 were made freshly and used in WB immediately to minimize protein degradation.



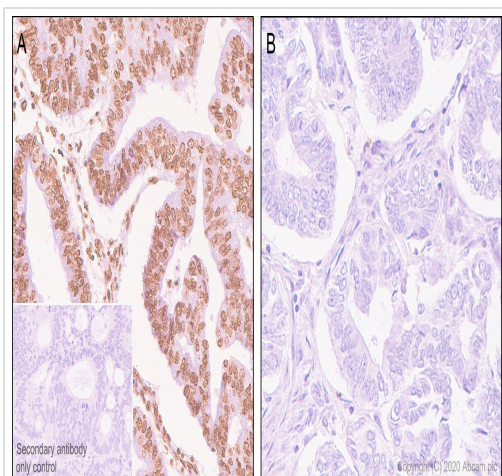
Dot blot analysis of STAT3 (phospho Y705) using ab267373 at 1/1000 followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1:100,000 dilution.

Lane 1: STAT3 phospho Y705 peptide (aa700-710)

Lane 2: Unmodified STAT3 peptide (aa698-710)

Exposure time: 3 minutes.

Blocking and diluting buffer and concentration: 5% NFDN/TBST.

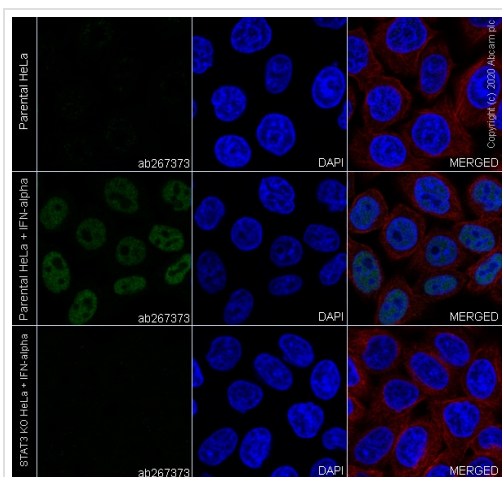


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue labeling STAT3 (phospho Y705) with ab267373 at 1/500 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)). Nuclear staining on human endometrial carcinoma without alkaline phosphatase treatment (image A) is observed. No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab267373 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

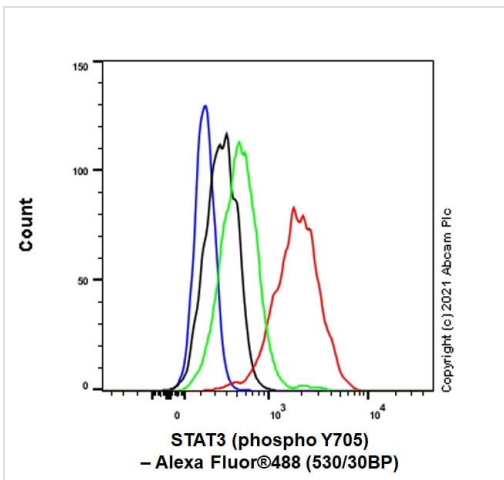


Immunocytochemistry/ Immunofluorescence - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 100% Methanol-permeabilized STAT3 KO HeLa cells labelling STAT3 (phospho Y705) with ab267373 at 1/500 dilution, followed by [ab150077](#) Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green).

Confocal image showing increased nuclear staining in parental HeLa cells treated with IFN-alpha (50 ng/ml) for 30 min, and no staining in STAT3 knockout HeLa cells treated with IFN-alpha (50 ng/ml) for 30 min.

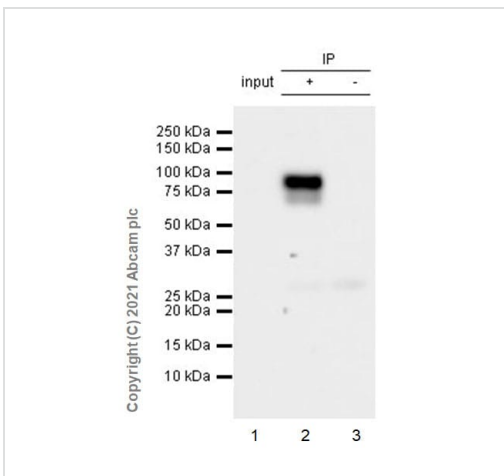
[ab195889](#) Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).



Flow Cytometry (Intracellular) - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Jurkat (Human T cell leukemia T lymphocyte) treated with 50 ng/ml IFN-alpha for 30min (Red)/ Untreated control (Green) cells labelling STAT3 (phospho Y705) with ab267373 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG ([ab172730](#)) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit IgG (Alexa Fluor® 488, [ab150077](#) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

STAT3 (phospho Y705) was immunoprecipitated from 0.35 mg Jurkat (human t cell leukemia cell line from peripheral blood) treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate 10 µg with ab267373 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab267373 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) was used at 1/5000 dilution.

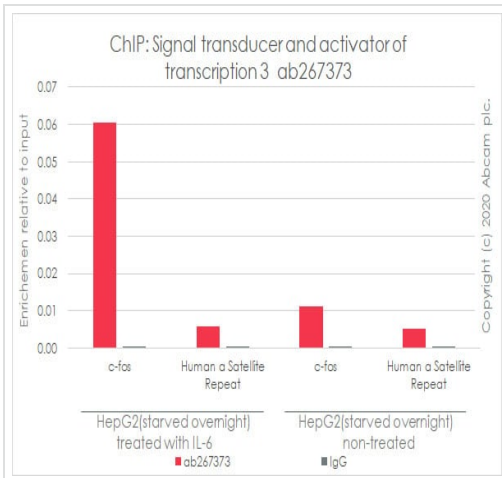
Lane 1: Jurkat treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate 10 µg

Lane 2: ab267373 IP in Jurkat treated with 50 ng/ml IFN alpha for 30 minutes whole cell lysate

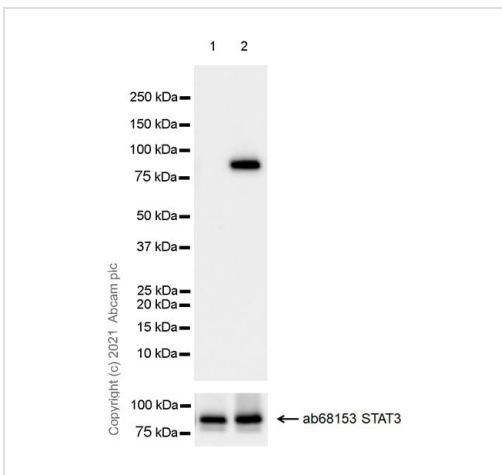
Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab267373 in Jurkat treated with 50 ng/ml IFN alpha for 30 minutes whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 15 seconds.



ChIP - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)



Western blot - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Chromatin was prepared from HepG2 (starved overnight) treated with IL-6 cells and HepG2 (starved overnight) non-treated according to the Abcam Dual-X-ChIP protocol*.

Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab267373 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers are purchased from competitor.

*[http://www.abcam.com/resources?](http://www.abcam.com/resources?keywords=X%20ChIP%20protocol)

keywords=X%20ChIP%20protocol

All lanes : Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373) at 1/1000 dilution

Lane 1 : HepG2 (human hepatocellular carcinoma epithelial cell) serum starved overnight, whole cell lysate

Lane 2 : HepG2 serum starved overnight, then treated with 100 ng/ml IL-6 for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 88 kDa

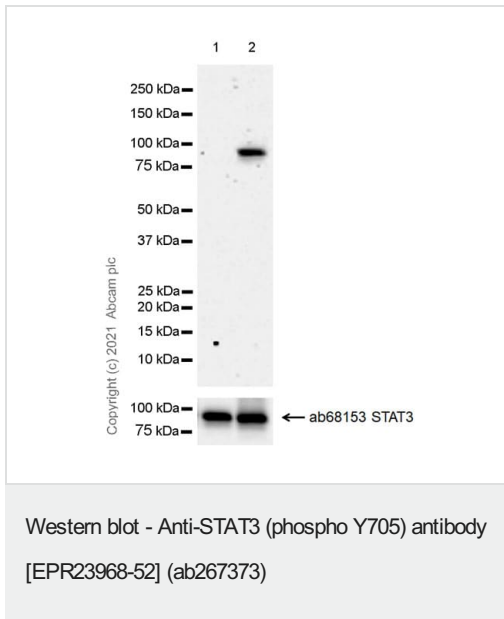
Observed band size: 88 kDa

Blocking and diluting buffer and concentration: 5% NFD/MBST.

IL-6 treatment induces phosphorylation of STAT3 at Tyr705 (PMID: 28676732).

Lysates were made freshly and used in WB immediately to minimize protein degradation.

Exposure time: 26 seconds.



All lanes : Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) serum starved overnight, whole cell lysate

Lane 2 : HeLa serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 88 kDa

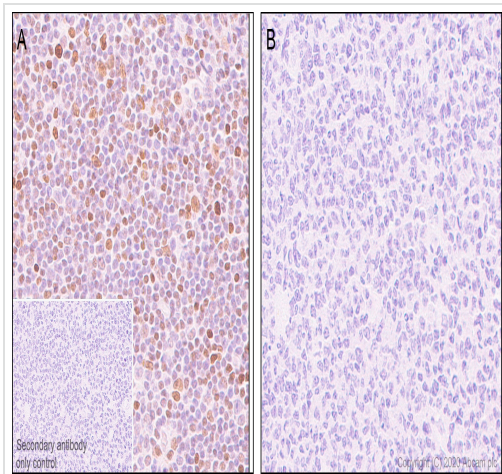
Observed band size: 88 kDa

Blocking and diluting buffer and concentration: 5% NFD/MTBST.

IFN alpha treatment induces phosphorylation of STAT3 at Tyr705 (PMID: 21957129).

Lysates were made freshly and used in WB immediately to minimize protein degradation.

Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (ab267373)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling STAT3 (phospho Y705) with ab267373 at 1/500 dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human tonsil without alkaline phosphatase treatment (image A) is observed. No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab267373 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

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