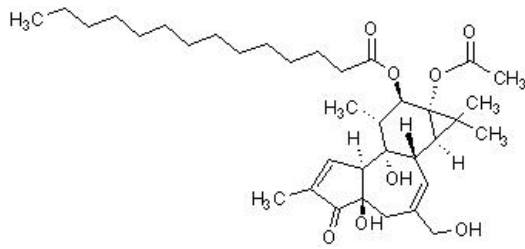


Product datasheet

Phorbol 12-myristate 13-acetate (PMA), PKC activator
ab120297

10 References 14 Images

Overview

Product name	Phorbol 12-myristate 13-acetate (PMA), PKC activator
Description	PKC activator
Biological description	Potent nanomolar activator of protein kinase C <i>in vivo</i> and <i>in vitro</i> . Binds to C1 domain of protein kinase C, induces membrane translocation and enzyme activation. Also reported to have actions on non-kinase proteins including chimaerins, RasGRP and Unc-13/Munc-13. Extremely potent mouse skin tumor promoter.
Purity	> 99%
CAS Number	16561-29-8
Chemical structure	

Properties

Chemical name	Phorbol 12-myristate 13-acetate
Molecular weight	616.83
Molecular formula	C ₃₆ H ₅₆ O ₈
PubChem identifier	27924
Storage instructions	Store at -20°C. Store under desiccating conditions. The product can be stored for up to 12 months.
Solubility overview	Soluble in DMSO to 100 mM and in ethanol to 10 mM
Handling	<p>This product is supplied in one (or more) pack size which is freeze dried. Therefore the contents may not be readily visible, as they can coat the bottom or walls of the vial. Please see our FAQs and information page for more details on handling.</p> <p>Wherever possible, you should prepare and use solutions on the same day. However, if you need to make up stock solutions in advance, we recommend that you store the solution as aliquots in tightly sealed vials at -20°C. Generally, these will be useable for up to one month. Before use, and</p>

prior to opening the vial we recommend that you allow your product to equilibrate to room temperature for at least 1 hour.

Need more advice on solubility, usage and handling? Please visit our [frequently asked questions \(FAQ\) page](#) for more details.

SMILES

CC(=O)O[C@@]43[C@H](OC(=O)CCCCCCCCCCCC)[C@@H](C)[C@@]1(O)[C@@H](C=C(CO)C[C@]2(O)C(=O)C(C)=C[C@@H]12)[C@@H]4C3(C)C

Source

Synthetic

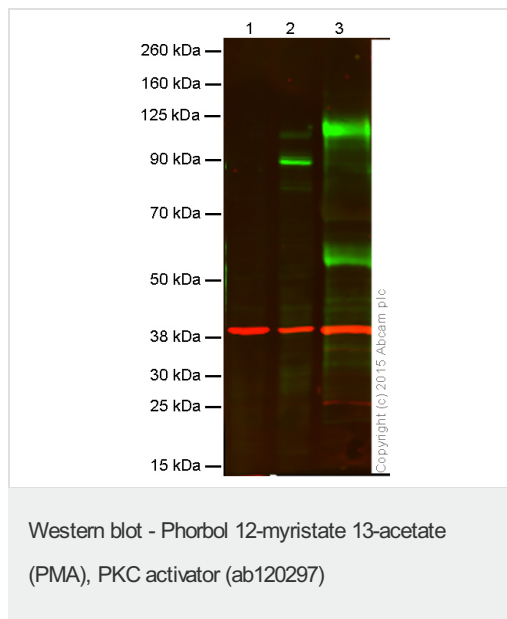
Applications

Our [Abpromise guarantee](#) covers the use of **ab120297** 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
Functional Studies		Use at an assay dependent concentration.

Images



All lanes : Anti-MMP9 antibody [EP1254] ([ab76003](#)) at 1.5 µg/ml

Lane 1 : Control U937 at 100 µg

Lane 2 : Stimulated U937 (24 hours with 10 ng x mL⁻¹ PMA (ab120297), 3 final hours with 3 ug x mL⁻¹ of Brefeldin ([ab120299](#))) at 100 µg

Lane 3 : Human tonsils at 20 µg

Secondary

All lanes : Goat anti-rabbit at 1/10000 dilution

Observed band size: 89 kDa

[why is the actual band size different from the predicted?](#)

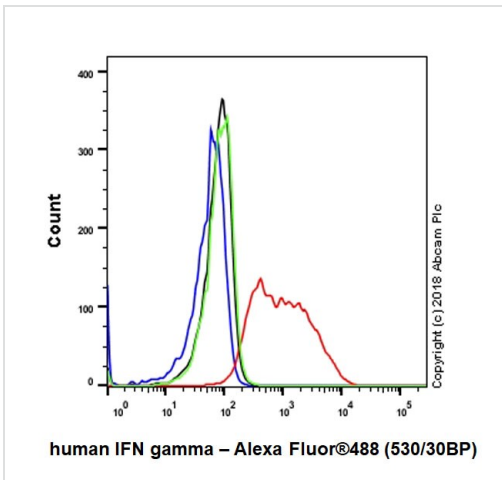
Running buffer: MOPS.

Conditions: Denatured/reduced.

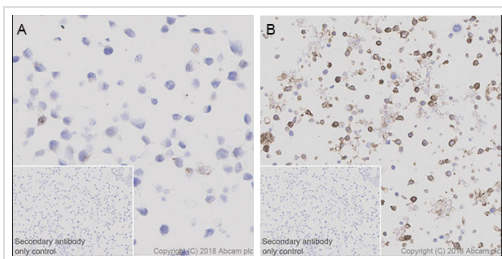
This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes.

The membrane was then blocked for an hour before being incubated with [ab76003](#) (rabbit-anti MMP9; 1.5 ug/mL) and [ab8245](#) (loading control to GAPDH; 0.1 ug/mL) for 48 hours at 4°C. Before

imaging, antibody binding was detected using infrared-labeled goat anti-rabbit (green) and goat anti-mouse (red) at 1:10,000 dilution for 1 hour at room temperature.



Flow Cytometry - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

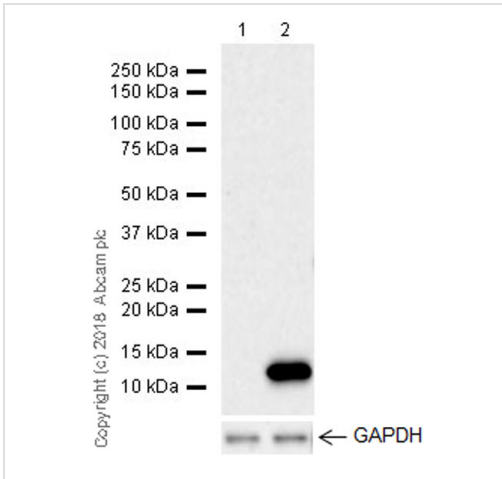


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NK-92 (human malignant non-Hodgkin's lymphoma natural killer cell) cell line treated with 80 μ M ab120297 PMA (Phorbol-12-myristate-13-acetate) and 3 μ M Ionomycin for 5 hours, then 300 ng/ml BFA was added for 4 hours labeling Interferon gamma with [ab231036](#) at 1/60 (red) and untreated control (green). Compared with a Rabbit monoclonal IgG-Isotype Control ([ab172730](#)) (black) and an unlabeled control (cells incubated with secondary antibody only) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, [ab150077](#)), at 1/2000 dilution was used as the secondary antibody.

Immunohistochemical analysis of paraffin-embedded NK-92 cells labeling Interferon gamma with [ab231036](#) at 1/500 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)). Nearly no staining on untreated NK92 cells (A) and positive staining on treated NK92 cells (B) (treated with 80 μ M ab120297 PMA (Phorbol-12-myristate-13-acetate) and 3 μ M Ionomycin for 5 hours, then with 300 ng/ml BFA for 4 hours) is observed (PMID:23129404). Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)).

Perform heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Western blot - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

All lanes : Anti-MCP1 antibody [EPR21025] (ab214819) at 1/1000 dilution

Lane 1 : Untreated THP-1 (human monocytic leukemia cell line) whole cell lysate

Lane 2 : THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours, then treated with 100ng/ml lipopolysaccharide (LPS) for 7 hours, then with 1 µg/ml Brefeldin A (BFA) added after 4 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

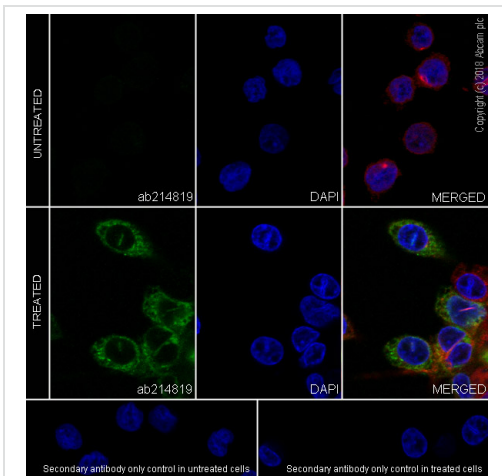
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 11 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

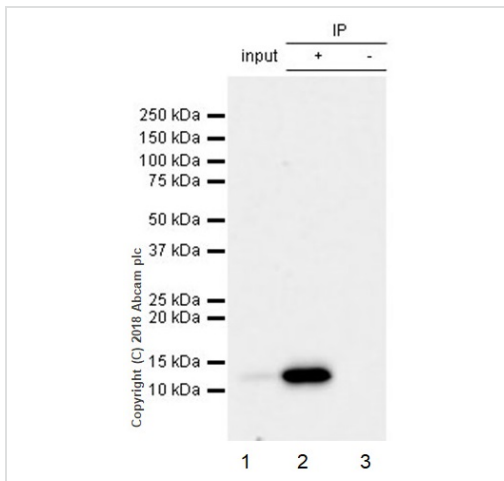


Immunocytochemistry/ Immunofluorescence - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized THP-1 (human monocytic leukemia cell line) cells, untreated or treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours, then treated with 100ng/ml lipopolysaccharide (LPS) for 7 hours, with 1 µg/ml Brefeldin A (BFA) added after 4 hours, labeling MCP1 with ab214819 at 1/50 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in THP-1 treated cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

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



Immunoprecipitation - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

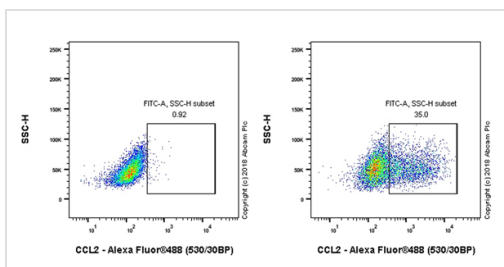
MCP1 was immunoprecipitated from 0.35 mg of THP-1 (human monocytic leukemia cell line) treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate with [ab214819](#) at 1/30 dilution. Western blot was performed from the immunoprecipitate using [ab214819](#) at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/1000 dilution.

Lane 1: THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate 10 µg (Input).

Lane 2: [ab214819](#) IP in THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate.

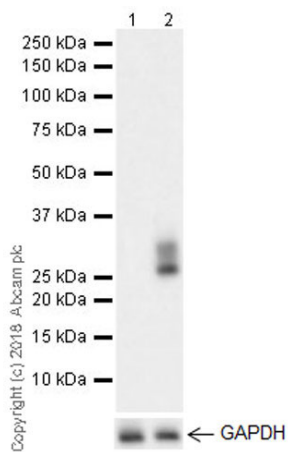
Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of [ab214819](#) in THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 0.1% Tween-20-permeabilized THP-1 (human monocytic leukemia cell line) cell line, treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h (Right) / Untreated control (Left) labeling MCP1 with [ab214891](#) at 1/500 dilution. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) at 1/2000 dilution was used as the secondary antibody.



Western blot - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

All lanes : Anti-CD69 antibody [EPR21814] ([ab233396](#)) at 1/5000 dilution

Lane 1 : Un-treated Daudi (human Burkitt's lymphoma lymphoblast) whole cell lysate

Lane 2 : Daudi treated with 50 ng/ml phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours

Lysates/proteins at 10 µg per lane.

Secondary

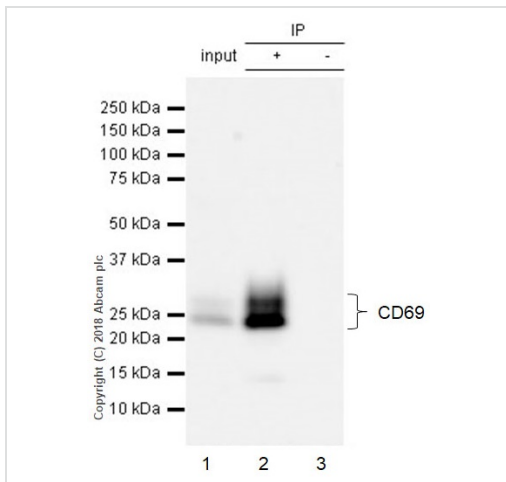
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Observed band size: 28,32 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 92 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

PMA treatment increases the basal level of p28/32 on Daudi. PMID: 1617156.



Immunoprecipitation - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

CD69 was immunoprecipitated from 0.35 mg Daudi (human Burkitt's lymphoma lymphoblast) treated with 50 ng/ml phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours whole cell lysate with [ab233396](#) at 1/30 dilution. Western blot was performed from the immunoprecipitate using [ab233396](#) at 1/5,000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/5,000 dilution.

Lane 1: Daudi (human Burkitt's lymphoma lymphoblast) treated with 50 ng/ml phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours whole cell lysate 10 µg (Input).

Lane 2: [ab233396](#) IP in Daudi treated with 50 ng/ml phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of [ab233396](#) in Daudi treated with 50 ng/ml phorbol-12-myristate-13-acetate (PMA, ab120297) for 24 hours whole cell lysate (-).

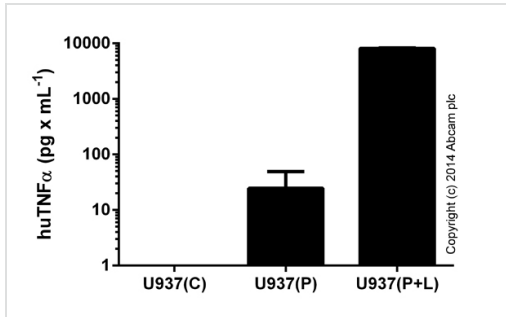
Blocking/Dilution buffer: 5% NFDm/TBST.



Functional Studies - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Serum starved HeLa cells were incubated at 37°C for 60 minutes with vehicle control (0 µM) and different concentrations of phorbol 12-myristate 13-acetate (PMA) (ab120297) in DMSO. Increased expression of PKC mu (phospho S916) ([ab81218](#)) correlates with an increase in phorbol 12-myristate 13-acetate (PMA) concentration, as described in literature.

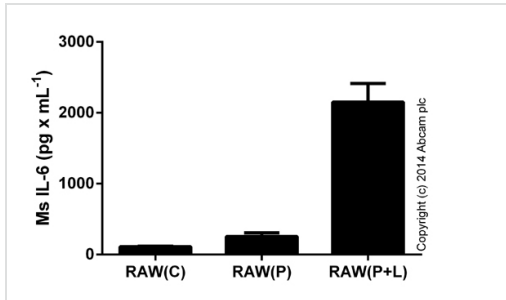
Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20 µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 3% milk before being incubated with [ab81218](#) at 1 µg/ml and [ab8227](#) at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP ([ab97051](#)) at 1/10000 dilution and visualised using ECL development solution.



Functional Studies - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Sandwich ELISA - TNF alpha Human ELISA Kit (ab100654)

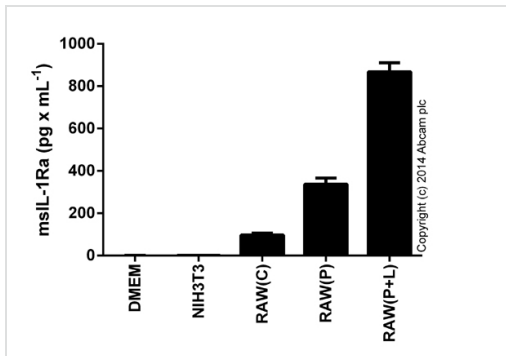
TNF α detected in supernatants from control cells (C) or cells stimulated for 24 hours with 50 ng x mL $^{-1}$ of PMA (ab120297) (P), and PMA with the addition of 1 μ g x mL $^{-1}$ of LPS (Sigma) (P+L) for the last 6 hours. Results shown after background signal was subtracted (duplicates +/- SD).



Functional Studies - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Sandwich ELISA - IL-6 (Interleukin-6) Mouse ELISA Kit (ab100712).

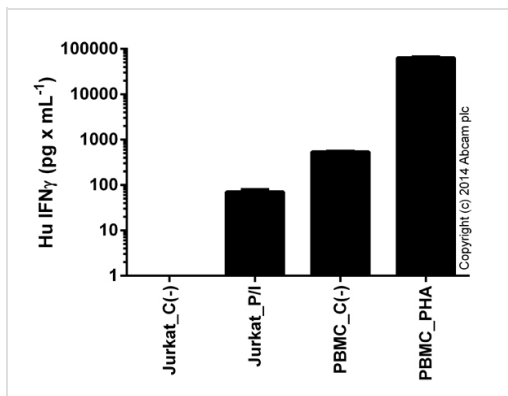
IL-6 detected in supernatants from RAW 246.7 control cells (C) or cells stimulated for 24 hours with 50 ng x mL $^{-1}$ of PMA (ab120297) (P), or 24 hours with PMA and 1 μ g x mL $^{-1}$ of LPS (Sigma) (P+L) for the last 6 hours. Results shown after background signal was subtracted (duplicates +/- SD).



Functional Studies - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Sandwich ELISA - IL-1ra (Interleukin-1ra) Mouse ELISA Kit (ab113348)

IL-1Ra detected in supernatants from RAW 246.7 control cells (C) or cells stimulated for 24 hours with 50 ng x mL $^{-1}$ of PMA (ab120297) (P), or 24 hours with PMA and 1 μ g x mL $^{-1}$ of LPS (Sigma) (P+L) for the last 6 hours. Results shown after background signal was subtracted (duplicates +/- SD).



Functional Studies - Phorbol 12-myristate 13-acetate (PMA), PKC activator (ab120297)

Sandwich ELISA - IFN gamma Human ELISA Kit (ab46025)

Jurkat were stimulated for 48 hours with 50 ng x mL $^{-1}$ of PMA (ab120297) and 1 μ M Ionomycin (ab120116) and PBMCs were stimulated for 48 hours with 2 % PHA-M (LifeTechnologies). Cell free supernatants were tested, showing results after background signal was subtracted (duplicates +/- SD).

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

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
- Abcam biochemicals are novel compounds and we have not tested their biological activity in house. Please use the literature to identify how to use these products effectively. If you require further assistance please contact the scientific support team