abcam

Product datasheet

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

46 References 13 图像

概述

化学结构

产品名称 Phorbol 12-myristate 13-acetate (PMA), PKC活化剂

描述 PKC活化剂 纯**度** > 98%

CAS编号 16561-29-8

10501-29-0

H₃C OH OH OH

性能

化学名称 Phorbol 12-myristate 13-acetate

分子量 616.83 分子式 C₃₆H₅₆O₈

PubChem识别号 27924

存放说明 Store at -20°C. Store under desiccating conditions. The product can be stored for up to 12

months.

溶解度概述 Soluble in DMSO to 100 mM and in ethanol to 10 mM

处理 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 $\underline{\textbf{FAQs}}$

and information page for more details on handling.

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 prior to opening the vial we recommend that you allow your product to equilibrate to room

temperature for at least 1 hour.

Refer to SDS for further information.

Need more advice on solubility, usage and handling? Please visit our frequently asked

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questions (FAQ) page for more details.

SMILES CC(=0)O[C@@]43[C@H](OC(=0)CCCCCCCCCCCCC)[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

来源 Synthetic

应用

The Abpromise guarantee Abpromise™承诺保证使用ab120297于以下的经测试应用

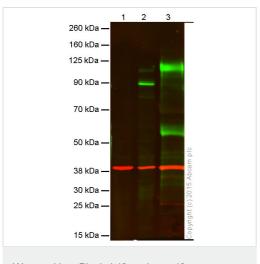
"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Functional Studies		Use at an assay dependent concentration.

图片

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

2D chemical structure image of ab120297, Phorbol 12-myristate 13-acetate (PMA), PKC activator



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

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 ug

Lane 3: Human tonsils at 20 µg

Secondary

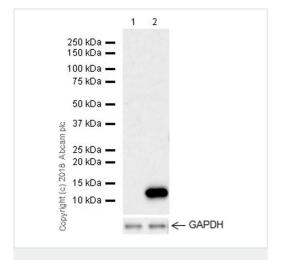
All lanes: Goat anti-rabbit at 1/10000 dilution

Observed band size: 89 kDa

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.



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

All lanes : Anti-MCP1 antibody [EPR21025] (<u>ab214819</u>) 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 μ g/ml Brefeldin A (BFA) added after 4 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Observed band size: 11 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

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 lgG 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.

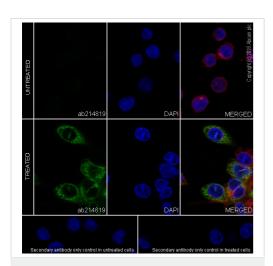
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

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.

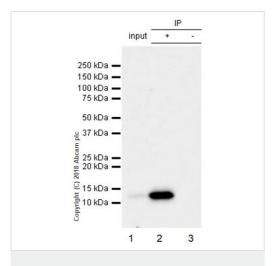
Lane 1: THP-1 treated with 80nM Phorbol-12-myristate-13-acetate (PMA, ab120297) for 24h, then treated with 100ng/ml lipopolysaccharide (LPS) for 4h, then together with 1µg/ml Brefeldin A (BFA) for another 3h whole cell lysate 10 µg (Input).

Lane 2: <u>ab214819</u> 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



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

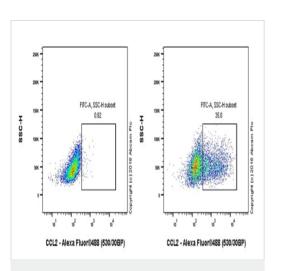


Immunoprecipitation - Phorbol 12-myristate 13acetate (PMA), PKC activator (ab120297)

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 1 μ g/ml Brefeldin A (BFA) for another 3h whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.

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.



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

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
37 kDa —
37 kDa —
37 kDa —
315 kDa —
4 kDZ (5) 110 kDa —
4 GAPDH

CAPACA —

GAPDH

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

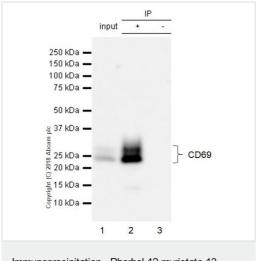
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 28,32 kDa

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 13acetate (PMA), PKC activator (ab120297)



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

CD69 was immunoprecipitated from 0.35 mg Daudi (human Burkitt's lymphoma lymphoblast) treated with 50 ng/ml phorbol-12myristate-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-mvristate-13-acetate (PMA, ab120297) for 24 hours whole cell lysate 10 µg (Input).

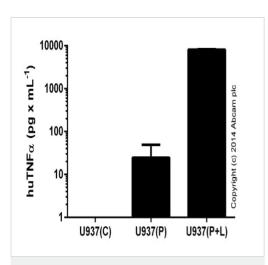
Lane 2: ab233396 IP in Daudi treated with 50 ng/ml phorbol-12myristate-13-acetate (PMA, ab120297) for 24 hours whole cell

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.

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.

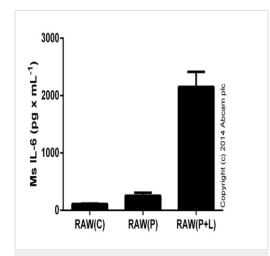
Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20 µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 3% milk before being incubated with ab81218 at 1 µg/ml 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.



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

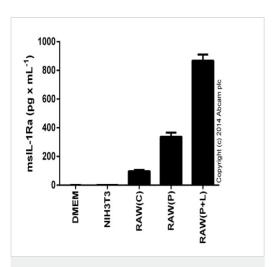
Sandwich ELISA - TNF alpha Human ELISA Kit (ab100654)

TNFa 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 ug x mL-1 of LPS (Sigma) (P+L) for the last 6 hours. Results shown after background signal was subtracted (duplicates +/- SD).



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

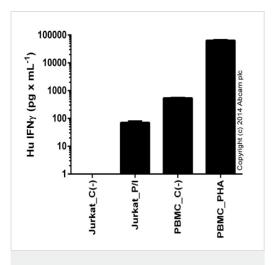
Sandwich ELISA - IL-6 (Interleukin-6) Mouse ELISA Kit (ab100712).



Sandwich ELISA - 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 ug x mL-1 of LPS (Sigma) (P+L) for the last 6 hours. Results shown after background signal was subtracted (duplicates +/- SD).



Sandwich ELISA - 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 lonomycin (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).

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