abcam

Product datasheet

Brefeldin A, Inhibitor of ADP-ribosylation factor ab120299

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概述

产品名称 Brefeldin A,抑制剂of ADP-ribosylation factor

描述 抑制剂of ADP-ribosylation factor

生物学描述 Reversible inhibitor of protein translocation from the endoplasmic reticulum to the Golgi

apparatus. Inhibits binding of the cytosolic coat protein, β-COP and ADP-ribosylation factor (ARF)

to Golgi membranes and inhibits GDP-GTP exchange.

CAS编号 20350-15-6

化学结构

HO ... HO ... H

性能

化学名称 (1*R*,2*E*,6*S*,10*E*,11a*S*,13*S*,14a*R*)-1,13-Dihydroxy-6-methyl-1,6,7,8,9,11a,12,13,14,14a-

decahydro-4H-cyclopenta[f]oxacyclotridecin-4-one

分子量 280.36

分子式 C₁₆H₂₄O₄

PubChem识别号 5287620

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 50 mM

处理 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.

Toxic, refer to SDS for further information.

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

questions (FAQ) page for more details.

SMILES O[C@@H]1C[C@H]2[C@H](O)C=CC(=O)O[C@@H](C)CCCC=C[C@@H]2C1

1

应用

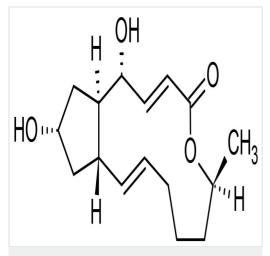
The Abpromise guarantee

Abpromise™承诺保证使用ab120299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。

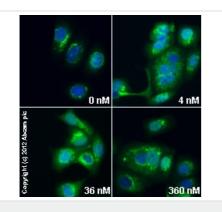
应用	Ab评论	说明
Functional Studies		Use at an assay dependent concentration.

图片



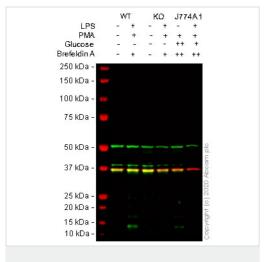
Chemical Structure - Brefeldin A, Inhibitor of ADP-ribosylation factor (ab120299)

2D chemical structure image of ab120299, Brefeldin A, Inhibitor of ADP-ribosylation factor



Immunocytochemistry/ Immunofluorescence -Brefeldin A, Inhibitor of ADP-ribosylation factor (ab120299) <u>ab84340</u> staining golgin-97 in MCF7 cells treated with brefeldin A (ab120299), by ICC/IF. Increase in Golgin-97 expression correlates with increased concentration of brefeldin A, as described in literature.

The cells were incubated at 37°C for 1.5 h in media containing different concentrations of ab120299 (brefeldin A) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab84340 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

All lanes : Anti-MCP3 antibody [EPR22649-155] (**ab228979**) at 1/1000 dilution

Lane 1 : Wild-type RAW 264.7 untreated control cell lysate

Lane 2 : Wild-type RAW 264.7 PMA treated (80 nM, 24 h) plus LPS treated (100 ng/ml, 6 h) and Brefeldin A (ab120299) treated (5 μ g/ml, 5 h) cell lysate

Lane 3: CCL7/MCP3 knockout RAW 264.7 untreated cell lysate

Lane 4: CCL7/MCP3 knockout RAW 264.7 PMA treated (80 nM, 24 h) plus LPS treated (100 ng/ml, 6 h) and Brefeldin A (ab120299) treated (5 μg/ml, 5 h) cell lysate

Lane 5 : J774A.1 Glucose treated (138.8 mMol/L, 8 h) plus Brefeldin A treated (5 μ g/ml, 6 h) and Brefeldin A (ab120299) treated (5 μ g/ml, 5 h) cell lysate

Lane 6 : J774A.1 Glucose treated (5.6 mMol/L, 8 h) plus Brefeldin A treated (5 μ g/ml, 6 h) and Brefeldin A (ab120299) treated (5 μ g/ml, 5 h) cell lysate

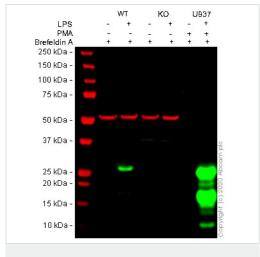
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 14 kDa

Lanes 1 - 6: Merged signal (red and green). Green - <u>ab228979</u> observed at 14 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab228979 was shown to react with MCP3 in RAW 264.7 wild-type cells in Western blot with loss of signal observed in CCL7 knockout sample. Wild-type and CCL7 knockout RAW 264.7 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab228979 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

All lanes : Anti-TNF alpha antibody [EPR22598-212] (**ab255275**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 Brefeldin A (ab120299) treated (5 μg/ml, 4 h) cell lysate

Lane 2: Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (ab120299) treated (5 µg/ml, 4 h) cell lysate

Lane 3: TNF alpha knockout THP-1 Brefeldin A (ab120299) treated (5 μg/ml, 4 h) cell lysate

Lane 4 : TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (ab120299) treated (5 μ g/ml, 4 h) cell lysate **Lane 5 :** U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A (ab120299) treated (5 μ g/ml, 4 h) cell lysate

Lane 6 : U937 PMA treated (10 mM, 2 days) and LPS treated (1 μ g/ml, 16 h) plus Brefeldin A (ab120299) treated (5 μ g/ml, 4 h) cell lysate

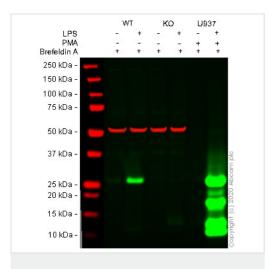
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 26 kDa

Lanes 1 - 6: Merged signal (red and green). Green - <u>ab255275</u> observed at 26 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab255275 was shown to react with TNF alpha in THP-1 wild-type cells in Western blot with loss of signal observed in TNF knockout sample. Wild-type and TNF knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab255275 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

All lanes : Anti-TNF alpha antibody [EPR19147] (ab183218) at 1/1000 dilution

Lane 1 : Wild-type THP-1 Brefeldin A (ab120299) treated (5 μg/ml, 4 h) cell lysate

Lane 2: Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (ab120299) treated (5 µg/ml, 4 h) cell lysate

Lane 3 : TNF alpha knockout THP-1 Brefeldin A (ab120299) treated (5 μ g/ml, 4 h) cell lysate

Lane 4 : TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (ab120299) treated (5 μg/ml, 4 h) cell lysate **Lane 5 :** U937 PMA treated (10 mM, 2 days) plus 16 h no

treatment and Brefeldin A (ab120299) treated (5 μ g/ml, 4 h) cell lysate

Lane 6 : U937 PMA treated (10 mM, 2 days) and LPS treated (1 $\mu g/ml,\,16$ h) plus Brefeldin A (ab120299) treated (5 $\mu g/ml,\,4$ h) cell lysate

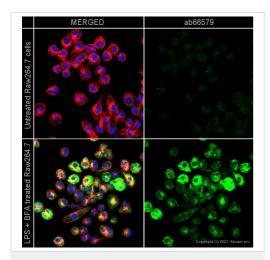
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

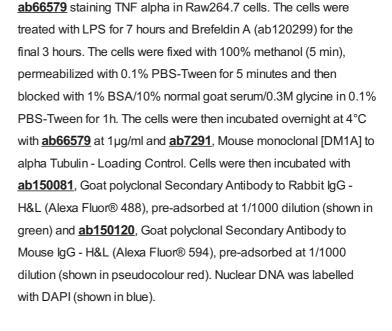
Observed band size: 26 kDa

Lanes 1 - 6: Merged signal (red and green). Green - <u>ab183218</u> observed at 26 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab183218 was shown to react with TNF alpha in THP-1 wild-type cells in Western blot with loss of signal observed in TNF knockout sample. Wild-type and TNF knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab183218 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry - Brefeldin A, Inhibitor of ADP-ribosylation factor (ab120299)



Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

All lanes : Anti-IP10 antibody [EPR20764] (**ab214668**) at 1/1000 dilution

Lane 1: Wild-type A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

 $\label{eq:Lane 2: Wild-type A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml, 32h), and Brefeldin A (ab120299)-treated (5 ug/ml for the last 6h) cell lysate$

Lane 3: IP10 knockout A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

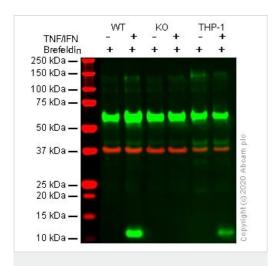
 $\label{eq:Lane 4: P10 knockout A549 IFN-y (ab259377) (100ng/ml, 32h)} $$ and TNF-alpha (ab259410) (10ng/ml, 32h), and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate$

Lane 5: THP-1 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

Lane 6: THP-1 IFN-y (ab259377) (200ng/ml, 24h) and LPS (50ng/ml, 24h)-treated for 24 hours, and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

Lysates/proteins at 30 μg per lane.

Performed under reducing conditions.

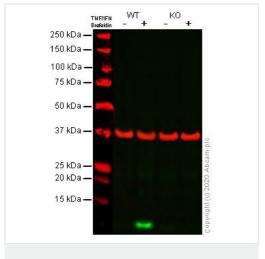


Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

Observed band size: 11 kDa

Lanes 1 - 6: Merged signal (red and green). Green - <u>ab214668</u> observed at 11 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab214668 was shown to react with IP10 in wild-type A549 cells in western blot with loss of signal observed in IP10 knockout cell line ab266971 (knockout cell lysate ab256888). Wild-type and IP10 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab214668 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216773) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

All lanes : Anti-IP10 antibody [EPR7850] (<u>ab137018</u>) at 1/500 dilution

Lane 1 : Wild-type A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

Lane 2: Wild-type A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml) for 32 hours, and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

Lane 3: IP10 knockout A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate

Lane 4: IP10 knockout A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml) for 32 hours, and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

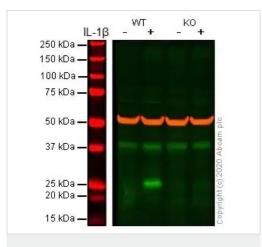
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 11 kDa

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab137018</u> observed at 11 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab137018 was shown to react with IP10 in A549 wild-type cells in western blot with loss of signal observed in IP10 knockout cell line ab266969 (IP10 knockout cell lysate ab256886). A549 wild-type and IP10 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab137018 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

All lanes : Anti-IL-6 antibody [EPR21711] (<u>ab233706</u>) at 1/1000 dilution

Lane 1 : Wild-type A549 Brefeldin A (ab120299)-treated (5ug/ml, 4h) cell lysate

 $\textbf{Lane 2:} \ \ \text{Wild-type A549 IL-1} \ \ (\underline{ab259387}) \ \ \ (20 \ \text{ng/ml, 24h}) \ \text{and}$ $\ \ \text{Brefeldin A (ab120299)-treated (5 \ \text{ug/ml for the last 4h}) \ \text{cell lysate}$

Lane 3: IL-6 knockout A549 Brefeldin A (ab120299)-treated (5ug/ml, 4h) cell lysate

 $\textbf{Lane 4: } \textbf{L-6} \text{ knockout A549 } \textbf{L-1} \textbf{(} \underline{\textbf{ab259387}} \textbf{) (20 ng/ml, 24h) and } \\ \textbf{Brefeldin A (ab120299)-treated (5 ug/ml for the last 4h) cell lysate }$

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

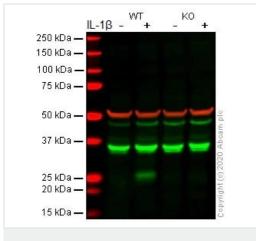
Observed band size: 25 kDa

Additional bands at: 40 kDa (possible non-specific binding)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab233706</u> observed at 25 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

<u>ab233706</u> was shown to react with IL-6 in wild-type A549 cells in western blot with loss of signal observed in IL-6 knockout cell line <u>ab273751</u> (knockout cell lysate <u>ab275501</u>). Wild-type and IL-6 knockout A549 cell lysates were subjected to SDS-PAGE.

Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with <u>ab233706</u> and <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Brefeldin A, Inhibitor of ADPribosylation factor (ab120299)

All lanes : Anti-IL-6 antibody [EPR20653] (<u>ab214429</u>) at 1/1000 dilution

Lane 1: Wild-type A549 Brefeldin A (ab120299)-treated (5ug/ml, 4h) cell lysate

Lane 2: Wild-type A549 IL-1ß (ab259387) (20 ng/ml, 24h) and Brefeldin A (ab120299)-treated (5 ug/ml for the last 4h) cell lysate

Lane 3: IL-6 knockout A549 Brefeldin A (ab120299)-treated (5ug/ml, 4h) cell lysate

 $\textbf{Lane 4: } \textbf{L-6} \text{ knockout A549 } \textbf{L-1} \textbf{(ab259387)} \text{ (20 ng/ml, 24h) and } \\ \textbf{Brefeldin A (ab120299)-treated (5 ug/ml for the last 4h) cell lysate }$

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab214429</u> observed at 25 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

<u>ab214429</u> was shown to react with IL-6 in wild-type A549 cells in western blot with loss of signal observed in IL-6 knockout cell line <u>ab273751</u> (knockout cell lysate <u>ab275501</u>). Wild-type A549 and IL-6 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with <u>ab214429</u> and <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

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