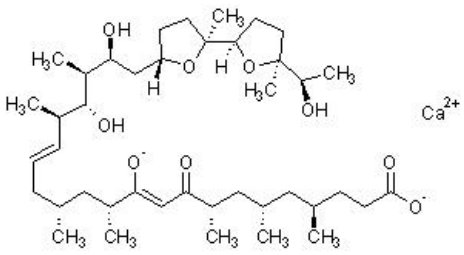
abcam

Product datasheet

Ionomycin Ca2+ Salt, Ca2+ ionophore ab120116

9 References 12 Images

Overview

Product name	Ionomycin Ca2+ Salt, Ca2+ ionophore
Description	Ca^{2+} ionophore
CAS Number	56092-82-1
Chemical structure	

Properties

Chemical name	(4*R*,6*S*,8*S*,10*Z*,12*R*,14*R*,16*E*,18*R*,19*R*,20*S*,21*S*)-11,19,21-Trihydroxy-4,6,8,12,14,18,20-heptamethyl-22-[(2*S*,2'*R*,5*S*,5'*S*)-octahydro-5'-[(1*R*)-1-hydroxyethyl]-2,5'-dimethyl[2,2'-bifuran]-5-yl]-9-oxo-10,16-docosadienoic acid calcium salt
Molecular weight	747.08
Molecular formula	$C_{41}H_{70}CaO_9$
PubChem identifier	6446270
Storage instructions	Store at +4°C. Store under desiccating conditions. The product can be stored for up to 12 months.
Solubility overview	Soluble in DMSO to 25 mM and in ethanol to 100 mM
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 questions (FAQ) page** for more details.
SMILES	[Ca+2].[O-]C(=O)CC[C@@H](C)C[C@H](C)C[C@H](C)C(=O)C=C(/[O-])[C@H](C)C[C@H]

(C)CC=C[C@@H](C)[C@@H](O)[C@@H](C)[C@@H](O)C[C@@H]1CC[C@](C)(O1)[C@H]2CC[C@](C)(O2)[C@@H](C)O

Source *Streptomyces conglobatus*

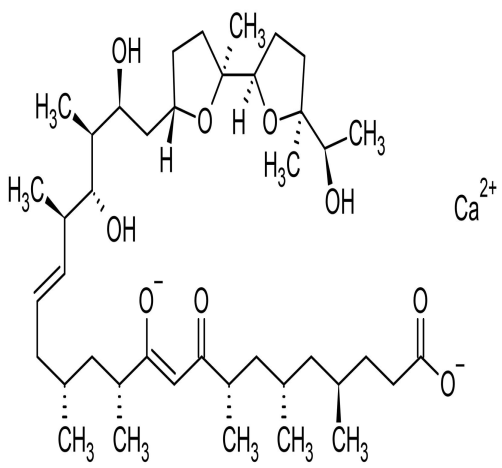
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab120116 in the following tested applications.

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

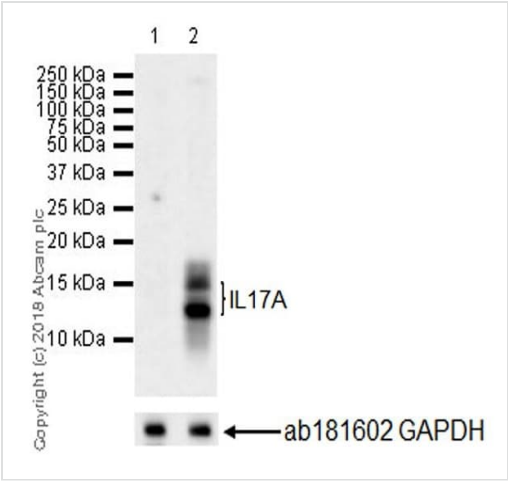
Application	Abreviews	Notes
Functional Studies		Use at an assay dependent concentration.

Images



Chemical Structure - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

2D chemical structure image of ab120116, Ionomycin Ca2+ Salt, Ca2+ ionophore



Western blot - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

All lanes : Anti-IL-17A antibody [EPR21776] (**ab218013**) at 1/1000 dilution

Lane 1 : Untreated EL4 (mouse lymphoma T lymphocyte) whole cell lysate
Lane 2 : EL4 treated with 50 ng/ml Phorbol-12-myristate-13-acetate (PMA) and 500 ng/ml ionomycin (ab120116) for 6 hours, then with 500 ng/ml Brefeldin A for 18 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

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

Developed using the ECL technique.

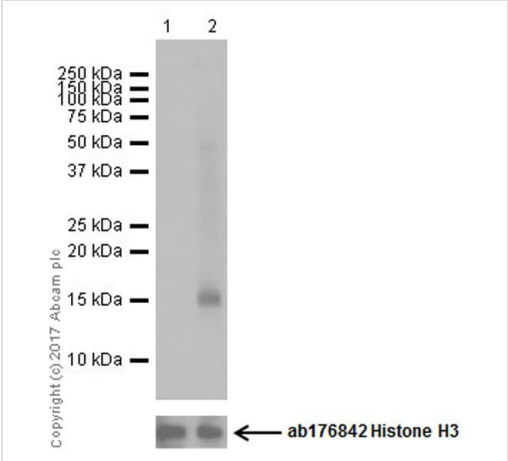
Observed band size: 14,17 kDa

Exposure time: 3 minutes

Blocking/dilution buffer and concentration: 5% NFDM/TBST

Expression of IL-17A can be induced by PMA and Ionomycin treatment (PMID 28382171).

The expression profile is consistent with the literature (PMID 9764847).



Western blot - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

All lanes : Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (**ab219406**) at 1/10000 dilution

Lane 1 : Whole cell lysate from HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours
Lane 2 : Whole cell lysate from HEK-293T cells transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at

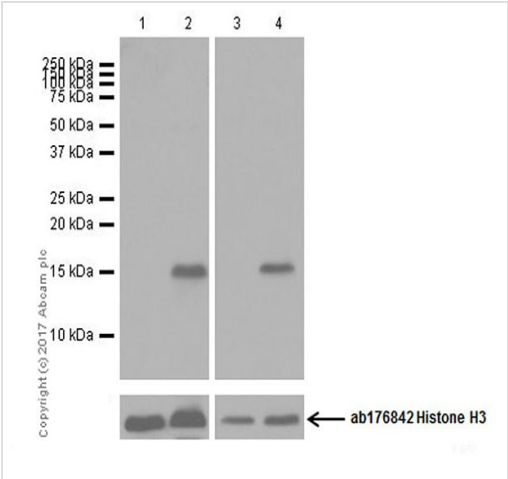
1/100000 dilution

Observed band size: 15 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST.

Histone H3R8 is citrullinated by PADI4 and $CaCl_2$ is used as a cofactor according to the literature (PMID: 16567635). Ionomycin is used to improve the modification by PADI4 according to the literature (PMID: 26360112).



Western blot - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

All lanes : Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (**ab219406**) at 1/5000 dilution

Lane 1 : Whole cell lysate from NIH/3T3 (Mouse embryonic fibroblast cell line) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 for 2 hours
Lane 2 : Whole cell lysate from NIH/3T3 transfected with PADI4 (WT) then treated with 10mM CaCl2 for 2 hours
Lane 3 : Whole cell lysate from C6 (Rat glial tumor cell line) transfected with empty vector with GFP tag (vector control) then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours
Lane 4 : C6 transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours

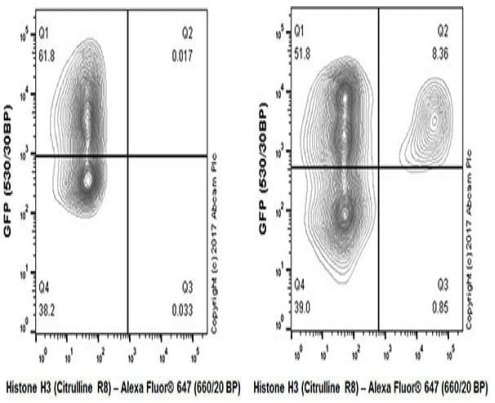
Lysates/proteins at 10 µg per lane.

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

Observed band size: 15 kDa

Exposure time: 1 second

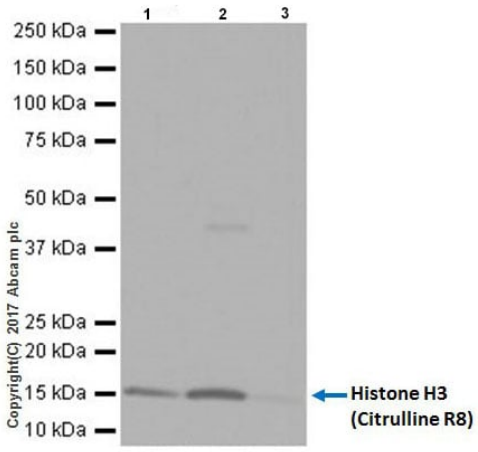
Blocking/Dilution buffer: 5% BSA/TBST.



Flow Cytometry (Intracellular) - Ionomycin Ca2+ Salt, Ca2+ ionophore (ab120116)

Flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector (left panel) or PADI4 (WT, right panel), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, labeling Histone H3 (citrulline R8) with **ab219406** at 1/500 dilution, followed by Goat anti rabbit IgG (Alexa Fluor® 647) at 1/2000 dilution.

Positive signal is obtained from HEK-293T cells transfected with WT PADI4 treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours.



Immunoprecipitation - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

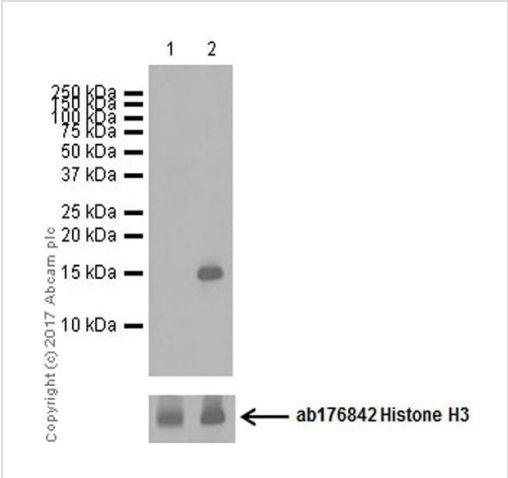
Histone H3 (citrulline R8) was immunoprecipitated from 0.35 mg of HEK-293T (Human epithelial cell line from embryonic kidney) transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate with **ab219406** at 1/30 dilution.

Western blot was performed from the immunoprecipitate using **ab219406** at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate 10 µg (Input).
Lane 2: **ab219406** IP in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab219406** in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

All lanes : Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (**ab219407**) at 1/5000 dilution

Lane 1 : HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate
Lane 2 : HEK-293T (Human epithelial cell line from embryonic kidney) transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

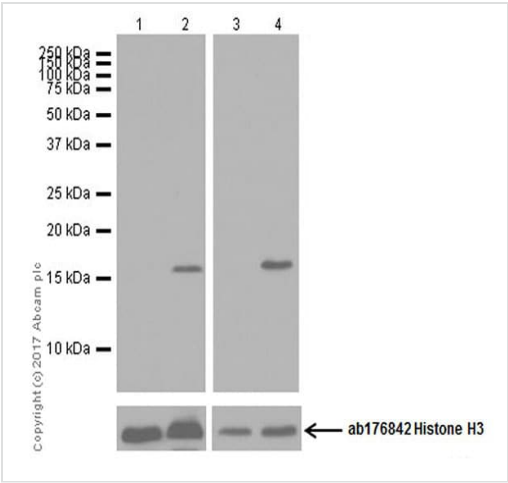
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Observed band size: 15 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% BSA/TBST.

Histone H3R17 is citrullinated by PADI4 and $CaCl_2$ is used as a cofactor according to the literature (PMID: 16567635). Ionomycin is used to improve the modification by PADI4 according to the literature (PMID: 26360112).



Western blot - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

All lanes : Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (**ab219407**) at 1/5000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast cell line) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 for 2 hours, whole cell lysate
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Lane 3 : C6 (Rat glial tumor cell line) transfected with empty vector with GFP tag (vector control) then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate
Lane 4 : C6 (Rat glial tumor cell line) transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate

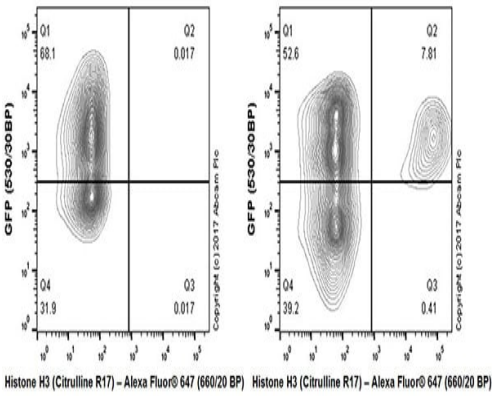
Lysates/proteins at 10 µg per lane.

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

Observed band size: 15 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST.



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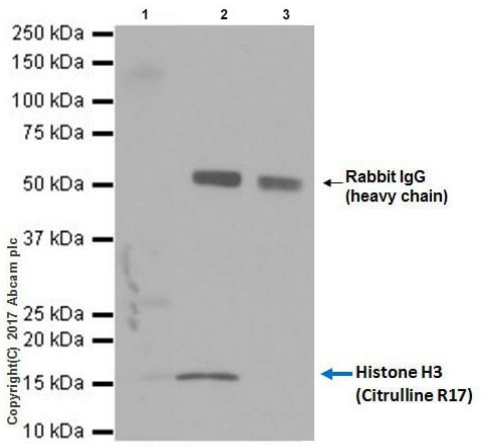
Positive signal is obtained from HEK-293T cells transfected with WT PADI4 treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours.



Immunoprecipitation - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

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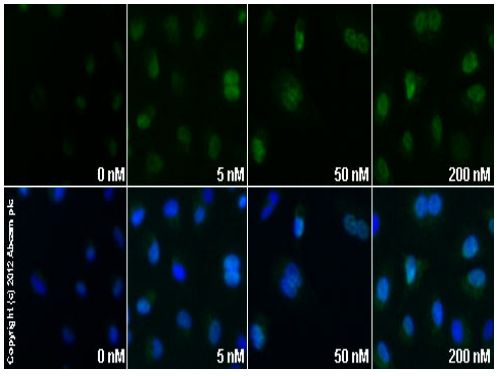
Western blot was performed from the immunoprecipitate using **ab219407** at 1/1000 dilution.

VeriBlot for IP secondary antibody (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate 10 µg (Input).
Lane 2: **ab219407** IP in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab219407** in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate.

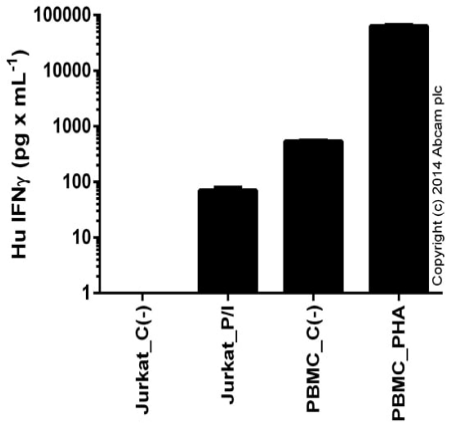
Blocking/Dilution buffer: 5% NFDM/TBST.



Functional Studies - Ionomycin Ca2+ Salt, Ca^{2+} ionophore (ab120116)

ab58668 staining ATF3 in A549 cells treated with ionomycin Ca^{2+} salt (ab120116), by ICC/IF. Increase in ATF3 expression correlates with increased concentration of ionomycin Ca^{2+} salt, as described in literature.

The cells were incubated at 37°C for 2h in media containing different concentrations of ab120116 (ionomycin Ca^{2+} salt) 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 **ab58668** (10 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-mouse polyclonal antibody (**ab96879**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Sandwich ELISA - Ionomycin Ca2+ Salt, Ca2+ ionophore (ab120116)

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 uM 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).

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