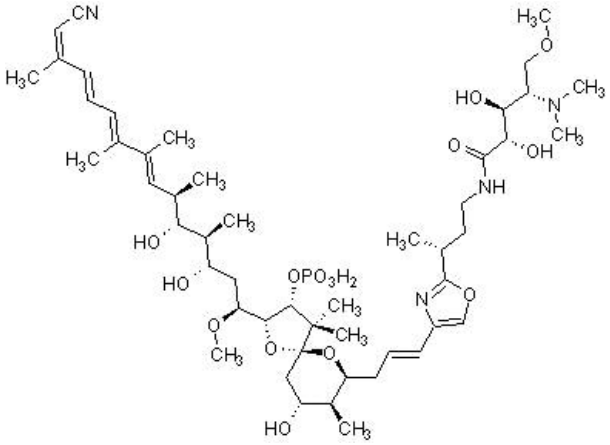
abcam

Product datasheet

Calyculin A, protein phosphatase inhibitor ab141784

8 References 8 Images

Overview

Product name	Calyculin A, protein phosphatase inhibitor
Description	Potent, selective and cell-permeable protein phosphatase inhibitor
Purity	> 98%
CAS Number	101932-71-2
Chemical structure	

Properties

Chemical name	[(2*R*,3*R*,5*R*,7*R*,8*S*,9*S*)-2-[(1*S*,3*S*,4*S*,5*R*,6*R*,7*E*,9*E*,11*E*,13*Z*)-14-Cyano-3,5-dihydroxy-1-methoxy-4,6,8,9,13-pentamethyltetradeca-7,9,11,13-tetraenyl]-9-[(*E*)-3-[2-[(2*S*)-4-[[(2*S*,3*S*,4*S*)-4-(dimethylamino)-2,3-dihydroxy-5-methoxypentanoyl]amino]butan-2-yl]-1,3-oxazol-4-yl]prop-2-enyl]-7-hydroxy-4,4,8-trimethyl-1,10-dioxaspiro[4.5]decan-3-yl] dihydrogen phosphate
Molecular weight	1009.18
Molecular formula	$C_{50}H_{81}N_4O_{15}P$
PubChem identifier	5311365
Storage instructions	Store at -20°C. Store under desiccating conditions. The product can be stored for up to 12 months.
Solubility overview	Soluble in ethanol and in DMSO
Handling	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.

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.

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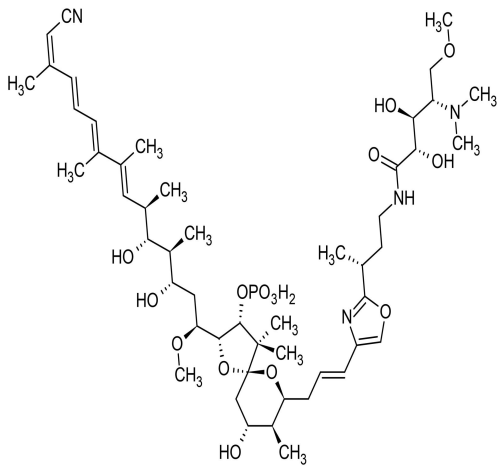
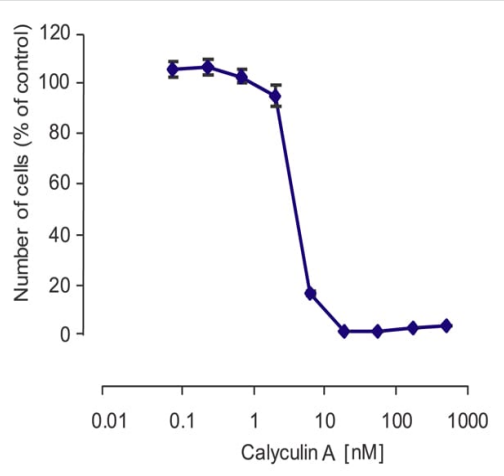
Source *Discodermia calyx*

Images



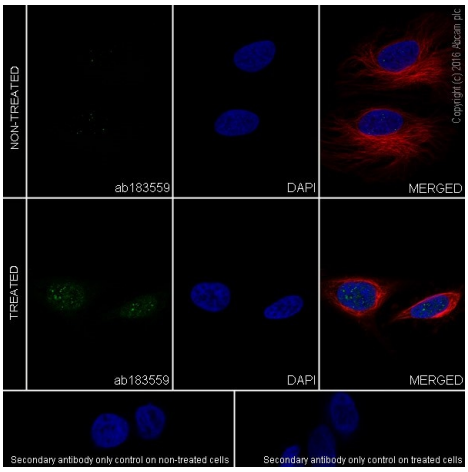
Chemical Structure - Calyculin A, protein phosphatase inhibitor (ab141784)

2D chemical structure image of ab141784, Calyculin A, protein phosphatase inhibitor



Functional Studies - Calyculin A, protein phosphatase inhibitor (ab141784)

Calyculin A inhibits the growth of breast cancer epithelial MCF7 cells. Cells were incubated with different concentrations of Calyculin A (ab141784) for four days. Cell number was measured using the methylene blue method. The number of cells was normalized with respect to the control (100%) and plotted against Calyculin A concentrations.



Immunocytochemistry/ Immunofluorescence - Calyculin A, protein phosphatase inhibitor (ab141784)

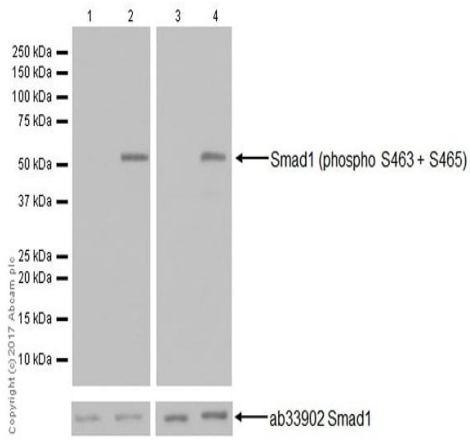
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling NF-kB p65 (phospho S276) with **ab183559** at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

The expression increased on HeLa cells after treatment with Calyculin A (ab141784 100ng/ml, 10min) then TNF-a (20ng/ml, 5min).

The nuclear counter stain is DAPI (blue).

Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Western blot - Calyculin A, protein phosphatase inhibitor (ab141784)

All lanes : Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-20] (**ab226821**) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) grown in serum-free media overnight, whole cell lysate
Lane 2 : HeLa grown in serum-free media overnight, then treated with 100 ng/ml Calyculin A (ab141784) for 15 minutes, followed by Calyculin A removal and treatment with 100 ng/ml BMP2 for 30 minutes, whole cell lysate
Lane 3 : NIH/3T3 (mouse embryo fibroblast cell line) grown in serum-free media overnight, whole cell lysate
Lane 4 : NIH/3T3 cultured in serum-free media overnight, then treated with 50 ng/ml BMP2 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 1/100000 dilution

Developed using the ECL technique.

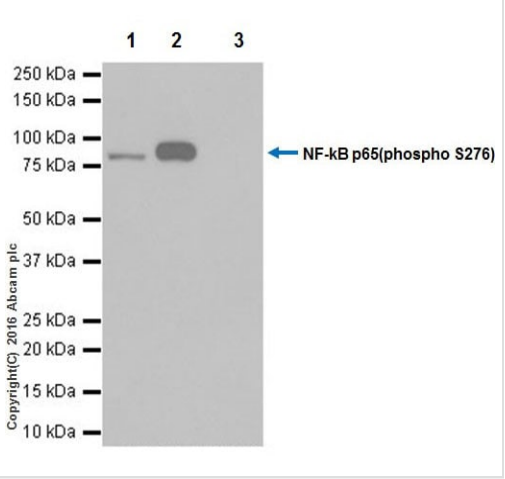
Observed band size: 60 kDa

Exposure time:

Lanes 1 and 2: 3 minutes.

Lanes 3 and 4: 30 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Calyculin A, protein phosphatase inhibitor (ab141784)

NF-kB p65 (phospho S276) was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysates, treated with 100ng/ml Calyculin A (ab141784) for 10min, then 20ng/ml TNA-a for 5min, with **ab183559** at 1/40 dilution. Western blot was performed from the immunoprecipitate using **ab183559** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

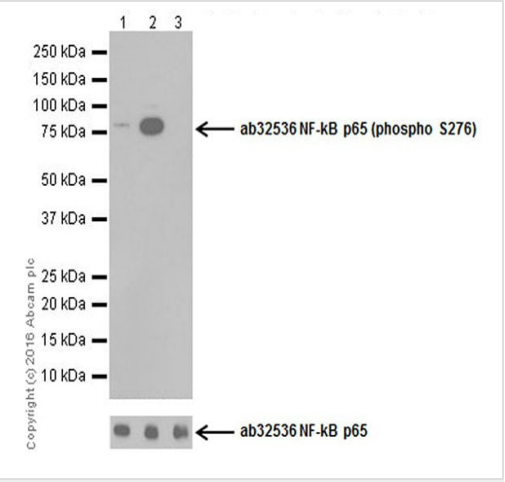
Lane 1: HeLa whole cell lysate treated with 100ng/ml Calyculin A (ab141784) for 10min, then 20ng/ml TNA-a for 5min, 10 µg (Input).

Lane 2: **ab183559** IP in HeLa whole cell lysate treated with 100ng/ml Calyculin A (ab141784) for 10min, then 20ng/ml TNA-a for 5min.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab183559** in HeLa whole cell lysate treated with 100ng/ml Calyculin A (ab141784) for 10min, then 20ng/ml TNA-a for 5min.

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

Exposure time: 10 seconds.



Western blot - Calyculin A, protein phosphatase inhibitor (ab141784)

All lanes : Anti-NF-kB p65 (phospho S276) antibody [EPR17622] (**ab183559**) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

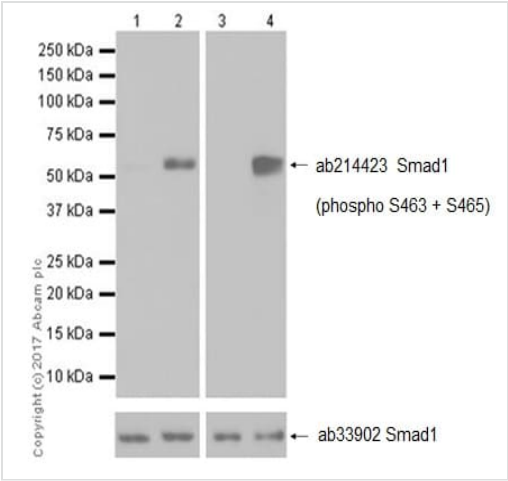
Lane 2 : HeLa whole cell lysate treated with 100ng/ml Calyculin A ab141784 for 30 minutes, then treated with 20ng/ml TNF-a for 5 minutes

Lane 3 : HeLa whole cell lysate treated with 100ng/ml Calyculin A ab141784 for 30 minutes, then treated with 20ng/ml TNF-a for 5 minutes, then treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 10 µg per lane.

Secondary

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



Western blot - Calyculin A, protein phosphatase inhibitor (ab141784)

All lanes : Anti-Smad1 (phospho S463 + S465) antibody [EPR20662-29] (**ab214423**) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) grown in serum-free media overnight, whole cell lysate
Lane 2 : HeLa grown in serum-free media overnight, then treated with 100ng/ml Calyculin A (ab141784) for 15 minutes, Calyculin A was removed, followed by treatment with 100ng/ml BMP2 for 30 minutes, whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast) grown in serum-free media overnight, whole cell lysate
Lane 4 : NIH/3T3 grown in serum-free media overnight, then treated with 50ng/ml BMP2 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 1/100000 dilution

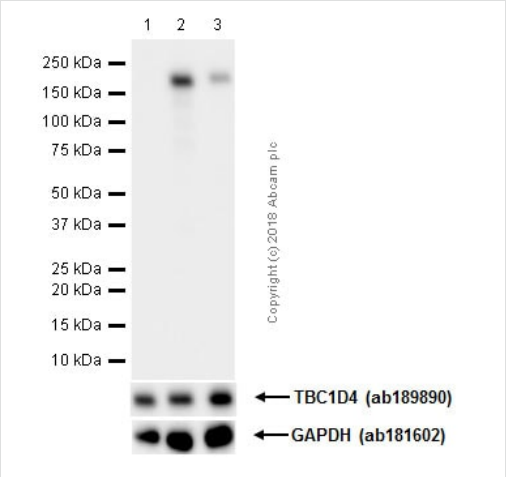
Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 60 kDa

Exposure time: 10 seconds

Blocking/Dilution: 5% NFDM/TBST.



Western blot - Calyculin A, protein phosphatase inhibitor (ab141784)

All lanes : Anti-AS160 (phospho T642) antibody [EPR2733(2)] (**ab131214**) at 1.12 µg/ml

Lane 1 : HEK-293 (human embryonic kidney epithelial cell) grown in serum free media overnight whole cell lysate
Lane 2 : HEK-293 grown in serum free media overnight, then treated with 100nM Calyculin A (ab141784) for 50min and then 100ng/ml Insulin was added for the last 20min, whole cell lysate
Lane 3 : HEK-293 grown in serum free media overnight, then treated with 100nM Calyculin A (ab141784) for 50min and then 100ng/ml Insulin was added for the last 20min, whole cell lysate. Then the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

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

Blocking and diluting buffer: 5% NFDM/TBST.

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