abcam

Product datasheet

Anti-DDIT3 antibody [9C8] - BSA and Azide free ab233121

敲除验证

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概述

产品名称	Anti-DDIT3抗体[9C8] - BSA and Azide free
描述	小鼠单克隆抗体[9C8] to DDIT3 - BSA and Azide free
宿主	Mouse
经测试应用	**适用于:** WB, ICC/IF
种属反应性	**与反应:** Mouse, Human **预测可用于:** Rat
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
表位	ab233121 has been shown to recognize an epitope in the N-terminal region of DDIT3.
阳性对照	WB: SW480 cell lysates, HeLa cells treated with 2ug/ml tunicamycin for 4 hours. ICC/IF: HeLa (untreated and tunicamycin-treated).
常规说明	ab233121 is the carrier-free version of **ab11419**. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or

contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at +4°C. Do Not Freeze.
存储溶液	Constituent: PBS
无载体	是
纯度	Protein G purified
克隆	单克隆
克隆编号	9C8
同种型	IgG2b
轻链类型	kappa

应用

The Abpromise guarantee Abpromise™承诺保证使用ab233121于以下的经测试应用

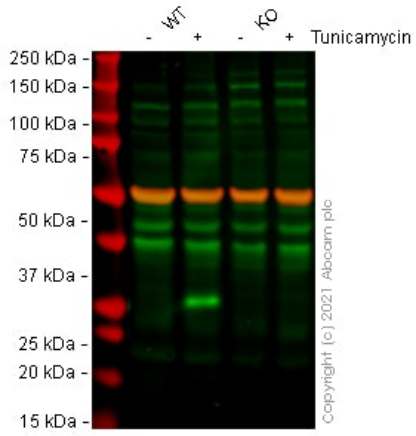
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use a concentration of 5 µg/ml. Detects a band of approximately 31 kDa (predicted molecular weight: 19 kDa). Please note that under normal cellular conditions this protein is not expressed in detectable levels, but is highly upregulated during times of cellular/ER stress. It is strongly recommended to run a positive control along your samples to confirm the expression levels of protein.
ICC/IF		Use a concentration of 5 µg/ml.

靶标

功能	Inhibits the DNA-binding activity of C/EBP and LAP by forming heterodimers that cannot bind DNA.
疾病相关	Note=A chromosomal aberration involving DDIT3 is found in a patient with malignant myxoid liposarcoma. Translocation t(12;16)(q13;p11) with FUS.
序列相似性	Belongs to the bZIP family. Contains 1 bZIP domain.
细胞定位	Nucleus.

图片



Western blot - Anti-DDIT3 antibody [9C8] - BSA and Azide free (ab233121)

All lanes : Anti-DDIT3 antibody [9C8] (**ab11419**) at 5 µg/ml

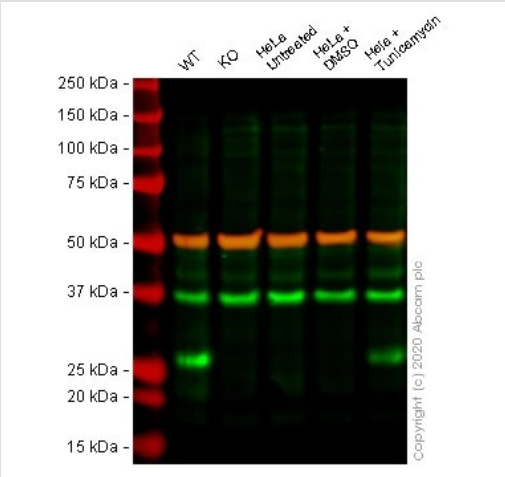
Lane 1 : Wild-type HeLa Vehicle Control Tunicamycin (20ug/mL, 4h) cell lysate
Lane 2 : Wild-type HeLa Treated Tunicamycin (20ug/mL, 4h) cell lysate
Lane 3 : DDIT3 knockout HeLa Vehicle Control Tunicamycin (20ug/mL, 4h) cell lysate
Lane 4 : DDIT3 knockout HeLa Treated Tunicamycin (20ug/mL, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 25 kDa

False colour image of Western blot: Anti-DDIT3 antibody [9C8] staining at 5 µg/ml, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab11419** was shown to bind specifically to DDIT3. A band was observed at 25 kDa in wild-type y cell lysates with no signal observed at this size in DDIT3 knockout cell line **ab265760** (knockout cell lysate **ab256889**). To generate this image, wild-type and DDIT3 knockout y cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution



Western blot - Anti-DDIT3 antibody [9C8] - BSA and Azide free (ab233121)

All lanes : Anti-DDIT3 antibody [9C8] (**ab11419**) at 5 µg/ml

Lane 1 : Wild-type SW480 cell lysate
Lane 2 : DDIT3 knockout SW480 cell lysate
Lane 3 : Untreated HeLa cell lysate
Lane 4 : HeLa + DMSO control cell lysate
Lane 5 : HeLa + tunicamycin (20ug/mL,4 hours) cell lysate

Lysates/proteins at 20 µg per lane.

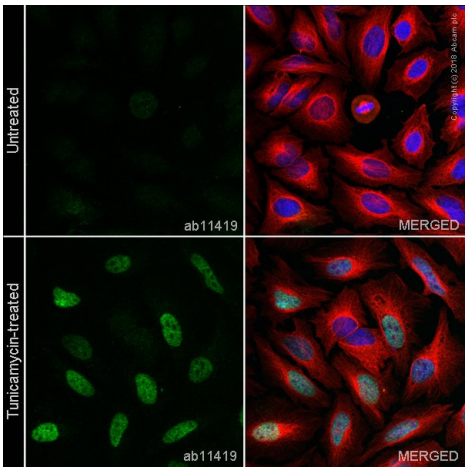
Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 26 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab11419**).

Lanes 1 - 5: Merged signal (red and green). Green - **ab11419** observed at 26 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab11419 was shown to react with DDIT3 in wild-type SW480 cells in western blot with loss of signal observed in DDIT3 knockout sample. Wild-type and DDIT3 knockout SW480 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab11419** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at 5 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



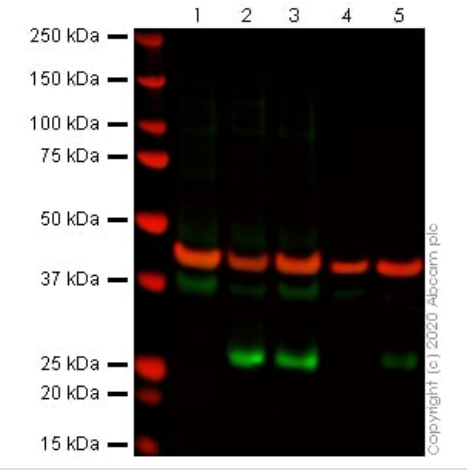
Immunocytochemistry/ Immunofluorescence - Anti-DDIT3 antibody [9C8] - BSA and Azide free (ab233121)

ab11419 staining DDIT3 in HeLa cells +/- Tunicamycin (1.5µM, 6 hours).

The cells were fixed with 4% PFA (10min), permeabilized with 0.1% Triton-X for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab11419** at 5µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (shown in green) and **ab150084**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, and sodium azide (**ab11419**).



Western blot - Anti-DDIT3 antibody [9C8] - BSA and Azide free (ab233121)

All lanes : Anti-DDIT3 antibody [9C8] (**ab11419**) at 5 µg/ml

Lane 1 : HeLa w/c control cell lysate at 40 µg
Lane 2 : HeLa cells treated with 2ug/ml tunicamycin for 4 hours, whole cell lysate cell lysate at 40 µg
Lane 3 : HeLa cells treated with 20ug/ml tunicamycin for 4 hours, whole cell lysate cell lysate at 40 µg
Lane 4 : HepG2 cell lysate at 20 µg
Lane 5 : NIH3T3 cell lysate at 20 µg

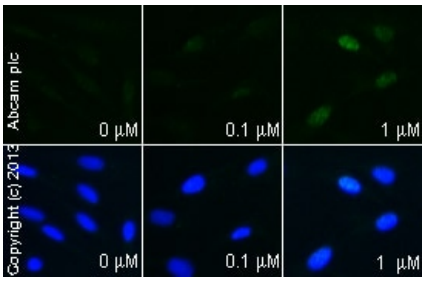
Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 27 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab11419**).

Lanes 1 - 5: Merged signal (red and green). Green - **ab11419** observed at 27 kDa. Red - loading control, Rabbit anti Actin observed at 42kDa.

ab11419 was shown to react with DDIT3 in western blot. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab11419** and Rabbit anti Actin overnight at 4°C at 5 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-DDIT3 antibody [9C8] - BSA and Azide free (ab233121)

ab11419 staining DDIT3 in SKNSH cells treated with deltamethrin (**ab141019**), by ICC/IF. Increase of DDIT3 expression correlates with increased concentration of deltamethrin, as described in literature.
The cells were incubated at 37°C for 48 hours in media containing different concentrations of **ab141019** (deltamethrin) in DMSO, fixed with 100% methanol for 5 minutes at -20°C 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 **ab11419** (10 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight® 488 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, and sodium azide (**ab11419**).

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