abcam

Product datasheet

Anti-DDIT3 antibody [9C8] ab11419

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31 Abreviews 220 References 8 Images

Overview

Product name	Anti-DDIT3 antibody [9C8]
Description	Mouse monoclonal [9C8] to DDIT3
Host species	Mouse
Tested applications	**Suitable for:** WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Epitope	ab11419 has been shown to recognize an epitope in the N-terminal region of DDIT3.
Positive control	WB: SW480 cell lysates, HeLa cells treated with 2ug/ml tunicamycin for 4 hours, NIH3T3 cell lysate, Wild-type HeLa Treated Tunicamycin cell lysate ICC/IF: HeLa (untreated and tunicamycin-treated).
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified

Clonality	Monoclonal
Clone number	9C8
Isotype	IgG2b
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab11419 in the following tested applications.

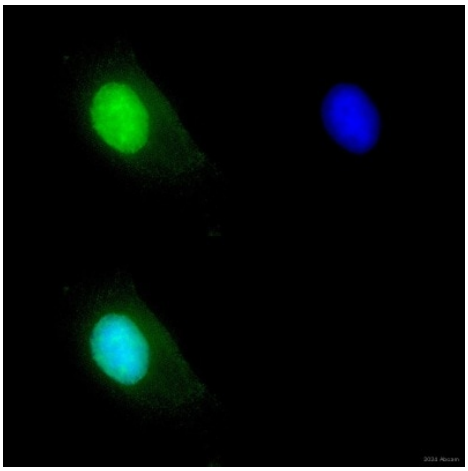
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ **(13)**	Use a concentration of 5 µg/ml. Detects a band of approximately 31 kDa (predicted molecular weight: 19 kDa). **DDIT3 is upregulated as a result of cellular or ER stress. It is strongly recommended to run a positive control (such as tunicamycin treated cell lysates) alongside your samples to confirm the protein expression level.** For blocking, we recommend using 3% milk for 1 hour. Please
ICC/IF	★★★★☆ **(5)**	Use a concentration of 5 µg/ml.

Target

Function	Inhibits the DNA-binding activity of C/EBP and LAP by forming heterodimers that cannot bind DNA.
Involvement in disease	Note=A chromosomal aberration involving DDIT3 is found in a patient with malignant myxoid liposarcoma. Translocation t(12;16)(q13;p11) with FUS.
Sequence similarities	Belongs to the bZIP family. Contains 1 bZIP domain.
Cellular localization	Nucleus.

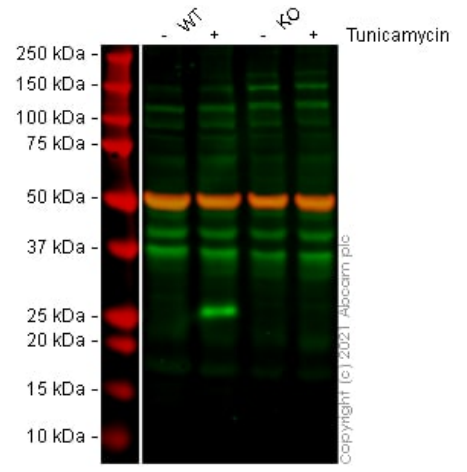
Images



Immunocytochemistry - Anti-DDIT3 antibody [9C8] (ab11419)

This image is courtesy of an anonymous Abreview

Immunocytochemistry analysis of formaldehyde-fixed HT29 cells permeabilized with 0.1% TritonX-100 in PBS for 10min staining with ab11419 at 1/500. Secondary antibody was ImmPRESS® HRP Goat Anti-Mouse IgG Polymer Detection. Samples were incubated with the primary antibody with Immunofluorescence Antibody Dilution Buffer for 18 hours at 4°C. Blocking was done using Peroxidase Blocking Solution, BLOXALL for 20 minutes at 20°C.



Western blot - Anti-DDIT3 antibody [9C8] (ab11419)

All lanes : Anti-DDIT3 antibody [9C8] (ab11419)

Lane 1 : Wild-type HeLa Vehicle Control Tunicamycin cell lysate
Lane 2 : Wild-type HeLa Treated Tunicamycin cell lysate
Lane 3 : DDIT3 knockout HeLa Vehicle Control Tunicamycin cell lysate
Lane 4 : DDIT3 knockout HeLa Treated Tunicamycin cell lysate

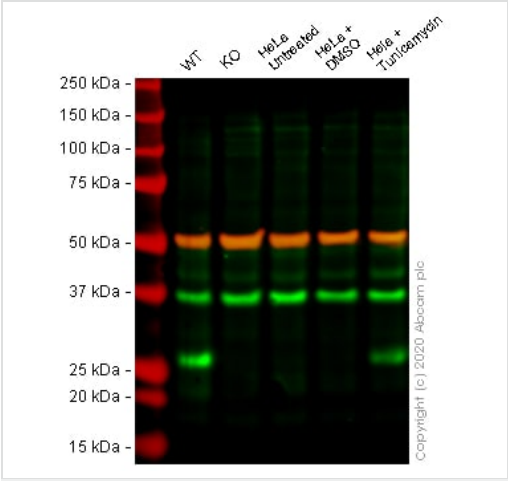
Lysates/proteins at 20 µg/ml per lane.

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 5µg/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] (ab11419)

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.

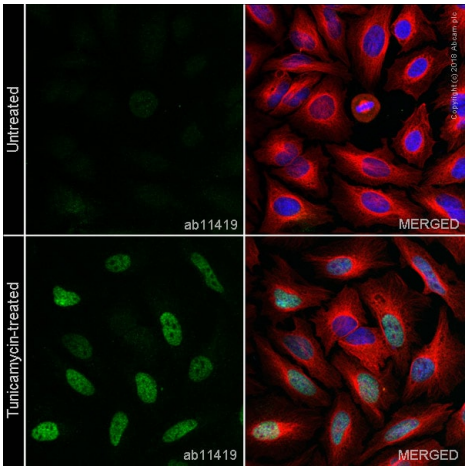
Performed under reducing conditions.

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

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.

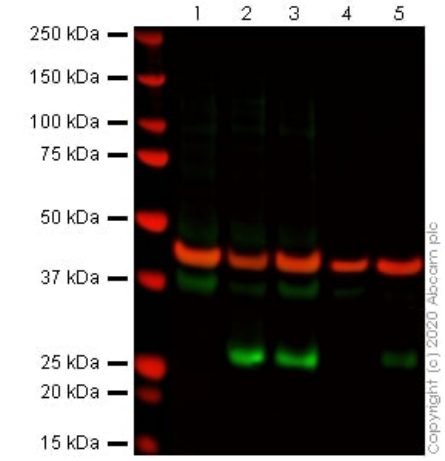


Immunocytochemistry/ Immunofluorescence - Anti-DDIT3 antibody [9C8] (ab11419)

ab11419 staining DDIT3 in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells +/- Tunicamycin 1.5µM, 6 hours (**ab120296**).

The cells were fixed with 4% PFA (10 min), permeabilized with 0.1% Triton-X for 5 mins 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 labeled with DAPI (shown in blue).

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



Western blot - Anti-DDIT3 antibody [9C8] (ab11419)

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

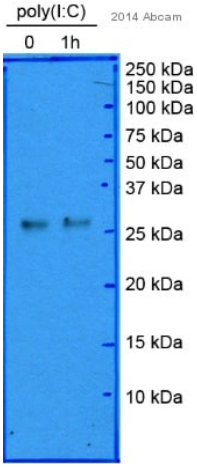
Performed under reducing conditions.

Predicted band size: 19 kDa

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.



Western blot - Anti-DDIT3 antibody [9C8] (ab11419)

This image is courtesy of an anonymous Abreview

All lanes : Anti-DDIT3 antibody [9C8] (ab11419) at 1/1000 dilution

All lanes : Mouse hepatocyte whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : HRP-conjugated goat anti-mouse IgG polyclonal at 1/10000 dilution

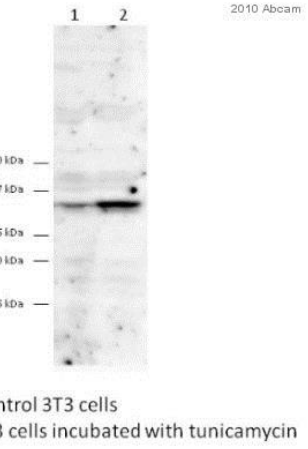
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 5 minutes

Treated with 20µg/ml poly(I:C).



Western blot - Anti-DDIT3 antibody [9C8] (ab11419)

This image is courtesy of an anonymous Abreview

All lanes : Anti-DDIT3 antibody [9C8] (ab11419) at 1/500 dilution (in TBST + 2.5% milk for 16 hours at 4°C)

Lane 1 : Whole cell lysate of Mouse 3T3 cells
Lane 2 : Whole cell lysate of Mouse 3T3 cells treated with tunicamycin for 24 hours

Lysates/proteins at 50 µg per lane.

Secondary

All lanes : An HRP-conjugated Goat anti-mouse IgG monoclonal at 1/2000 dilution

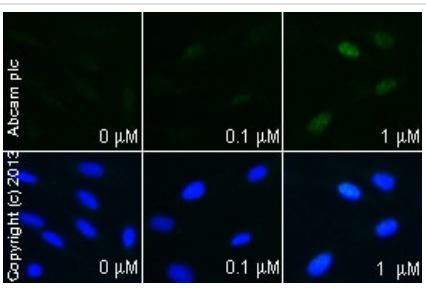
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 31 kDa

Exposure time: 2 minutes

Blocking Step: 5% Milk for 2 hours at 22°C



Immunocytochemistry/ Immunofluorescence - Anti-DDIT3 antibody [9C8] (ab11419)

ab11419 staining DDIT3 in SK-N-SH (human neuroblastoma cell line) 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.

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