abcam

Product datasheet

Anti-DDIT3 antibody [9C8] ab11419

KO VALIDATED

29 Abreviews 153 References 6 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	Other Immunogen Type corresponding to DDIT3. A bacterially expressed, mouse DDIT3 fusion protein.
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. ICC/IF: HeLa (untreated and tunicamycin-treated).
General notes	**Western blot protocol advice**: 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 see the WB image legend for more protocol information. This antibody clone is manufactured by Abcam. If you require a different buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work

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Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as 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

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.

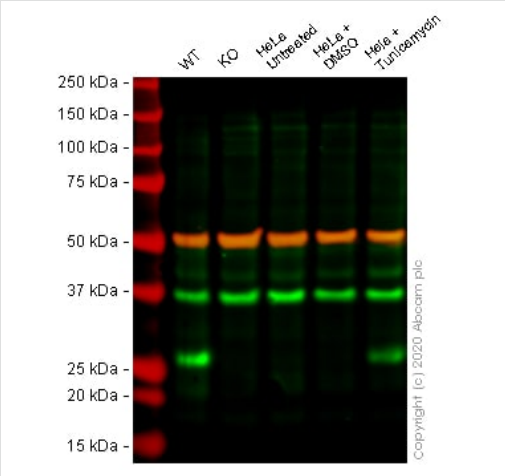
Application	Abreviews	Notes
WB	★★★★☆	Use a concentration of 5 µg/ml. Detects a band of approximately 31 kDa (predicted molecular weight: 19 kDa). **DDIT3 is upregulated as a result of cellular or ER stress. It is strongly recommended to run a positive control (such as tunicamycin treated cell lysates)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



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

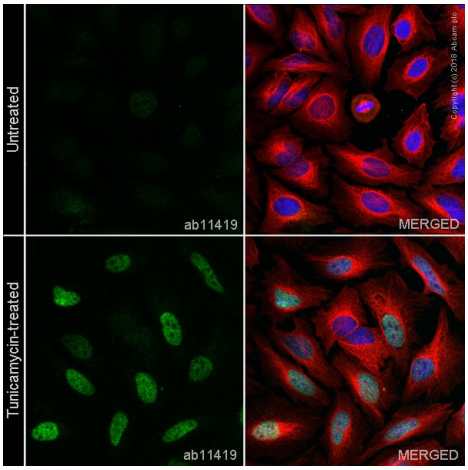
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 26 kDa
why is the actual band size different from the predicted?

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.

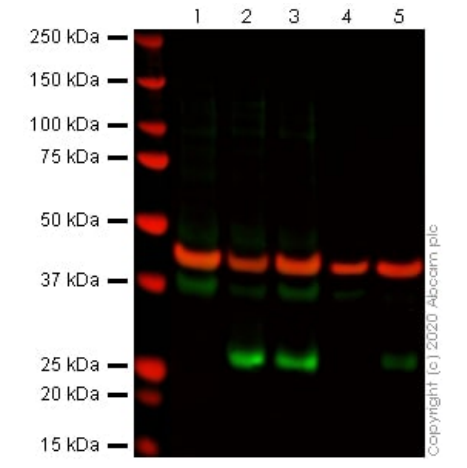


Immunocytochemistry/ Immunofluorescence - Anti-DDIT3 antibody [9C8] (ab11419)

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

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

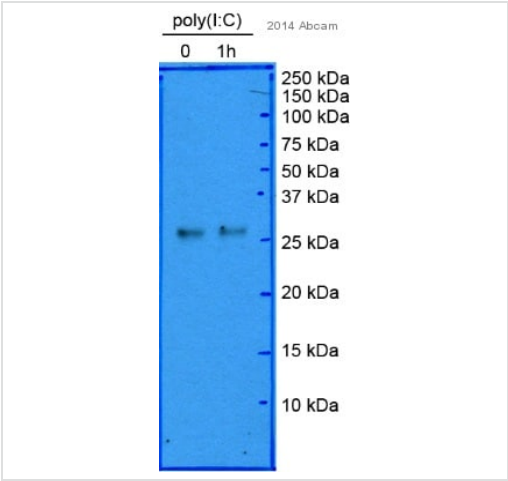
Performed under reducing conditions.

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.

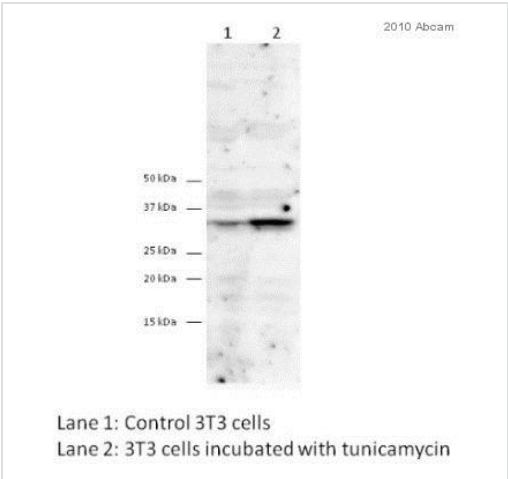
Performed under reducing conditions.

Predicted band size: 19 kDa

Observed band size: 27 kDa why is the actual band size different from the predicted?

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 lystate of Mouse 3T3 cells

Lane 2 : Whole cell lystate 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

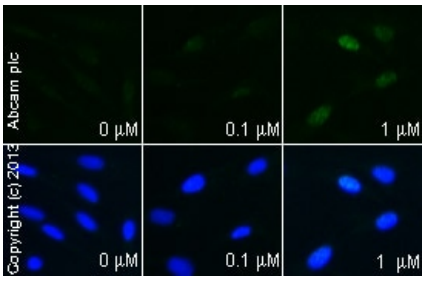
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 31 kDa why is the actual band size different from the predicted?

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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