abcam

Product datasheet

Anti-c-Fos antibody [2H2] ab208942



★★★★ 13 Abreviews 75 References 10 Images

Overview

Product name Anti-c-Fos antibody [2H2]

Description Mouse monoclonal [2H2] to c-Fos

Host species Mouse

Tested applications Suitable for: IHC-FrFI, IHC-P, WB, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow, Pig

Immunogen Recombinant full length protein corresponding to Human c-Fos aa 1 to the C-terminus. purified

from E. coli.

Database link: P01100

Run BLAST with Run BLAST with

Positive control IHC-P: Rat hippocampus, rat brain, rat placenta, human cervix and human placenta. WB: Rat

> cortical neurons treated with membrane depolarization buffer. HeLa cell lysate (serum-starved and then stimulated with 20% Fetal Bovine Serum for 2 hours). ICC/IF: Rat brain neural cultures.

HeLa cells, treated with 20% FBS for 2 hours following serum-starvation.

General notes 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 guestions, 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: 0.03% Sodium azide

Constituents: PBS, 50% Glycerol

Purity Affinity purified

Purification notes Affinity purified from tissue culture supernatant.

Clonality Monoclonal

Clone number 2H2 Isotype lgG1 Light chain type kappa

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab208942 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
IHC-FrFI	★★★ ☆☆ <u>(4)</u>	1/200.
IHC-P	★★★★★ (6)	1/1000.
WB		1/1000 - 1/2000. Predicted molecular weight: 41 kDa.
ICC/IF	★★★★☆ (1)	1/1000.

Target

Function

Nuclear phosphoprotein which forms a tight but non-covalently linked complex with the JUN/AP-1 transcription factor. In the heterodimer, FOS and JUN/AP-1 basic regions each seems to interact with symmetrical DNA half sites. On TGF-beta activation, forms a multimeric

SMAD3/SMAD4/JUN/FOS complex at the AP1/SMAD-binding site to regulate TGF-betamediated signaling. Has a critical function in regulating the development of cells destined to form and maintain the skeleton. It is thought to have an important role in signal transduction, cell proliferation and differentiation.

Sequence similarities

Belongs to the bZIP family. Fos subfamily.

Contains 1 bZIP domain.

Post-translational modifications

Phosphorylated in the C-terminal upon stimulation by nerve growth factor (NGF) and epidermal growth factor (EGF). Phosphorylated, in vitro, by MAPK and RSK1. Phosphorylation on both Ser-362 and Ser-374 by MAPK1/2 and RSK1/2 leads to protein stabilization with phosphorylation on Ser-374 being the major site for protein stabilization on NGF stimulation. Phosphorylation on Ser-362 and Ser-374 primes further phosphorylations on Thr-325 and Thr-331 through promoting docking of MAPK to the DEF domain. Phosphorylation on Thr-232, induced by HA-RAS, activates the transcriptional activity and antagonizes sumoylation. Phosphorylation on Ser-362 by

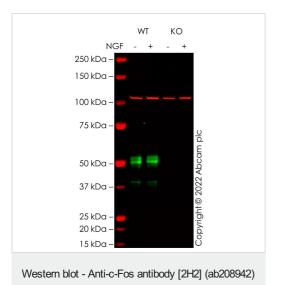
RSK2 in osteoblasts contributes to osteoblast transformation.

Constitutively sumoylated by SUMO1, SUMO2 and SUMO3. Desumoylated by SENP2. Sumoylation requires heterodimerization with JUN and is enhanced by mitogen stimulation. Sumoylation inhibits the AP-1 transcriptional activity and is, itself, inhibited by Ras-activated

phosphorylation on Thr-232.

Cellular localization

Nucleus.



All lanes: Anti-c-Fos antibody [2H2] (ab208942) at 1/1000 dilution

Lane 1: Wild-type PC-12 Untreated Control NGF (0 ng/mL, 4 days) cell lysate

Lane 2: Wild-type PC-12 Treated NGF (111 ng/mL, 4 days) cell lysate

Lane 3: FOS knockout PC-12 Untreated Control NGF (0 ng/mL, 4 days) cell lysate

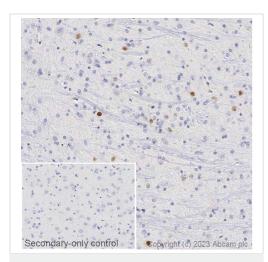
Lane 4: FOS knockout PC-12 Treated NGF (111 ng/mL, 4 days) cell lysate

Lysates/proteins at 20 µg per lane.

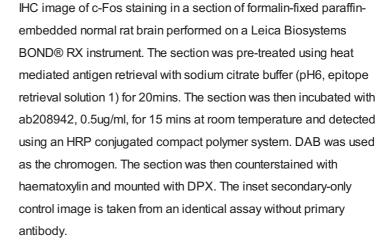
Performed under reducing conditions.

Predicted band size: 41 kDa **Observed band size:** 45-55 kDa

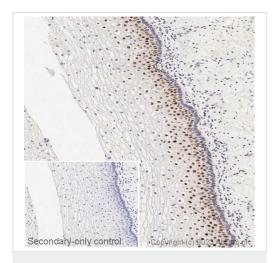
False colour image of Western blot: Anti-c-Fos antibody [2H2] staining at 1/1000 dilution, shown in green; Rabbit anti-ACTN4 [EPR2533(2)] (ab108198) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab208942 was shown to bind specifically to c-Fos. A band was observed at 45-55 kDa in treated wild-type PC-12 cell lysates with no signal observed at this size in FOS knockout cell line ab281615 (knockout cell lysate ab283111). Treatment of NGF has no observable affect on protein expression in this cell line. To generate this image, wild-type and FOS knockout PC-12 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Fos antibody [2H2] (ab208942)



For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

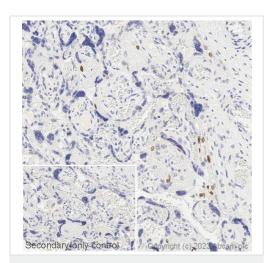


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Fos antibody [2H2] (ab208942)

IHC image of c-Fos staining in a section of formalin-fixed paraffinembedded normal human cervix* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab208942, 0.5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

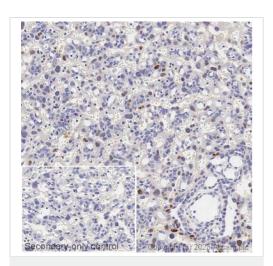


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Fos antibody [2H2] (ab208942)

IHC image of c-Fos staining in a section of formalin-fixed paraffinembedded normal human placenta* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab208942, 0.5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

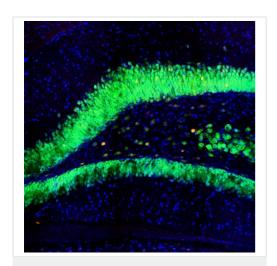
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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Fos antibody [2H2] (ab208942)

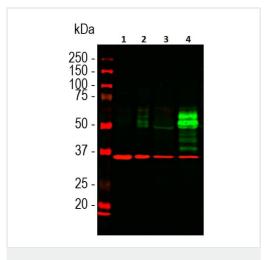
IHC image of c-Fos staining in a section of formalin-fixed paraffinembedded normal rat placenta performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab208942, 0.5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry - Free Floating - Anti-c-Fos antibody [2H2] (ab208942)

Section of rat hippocampus stained with ab208942, at a 1/200 dilution, in red and counterstained with rabbit polyclonal antibody to FOX3/NeuN. DAPI reveals nuclei of neurons and glia in blue. The hippocampal neurons stain green for FOX3/NeuN and a few also are expressing c-FOS, and so appear orange. These cells were spontaneously active at the time the animal was sacrificed.



Western blot - Anti-c-Fos antibody [2H2] (ab208942)

All lanes: Anti-c-Fos antibody [2H2] (ab208942) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cells in serum free media

Lane 2: HeLa cells stimulated with 20% fetal bovine serum for 2hrs after 36hrs in serum free media

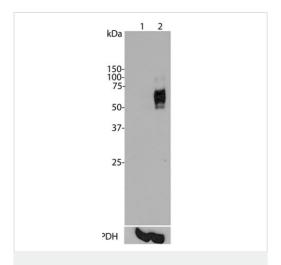
Lane 3: Rat cortical neurons

Lane 4 : Rat cortical neurons treated with membrane depolarization buffer for 5hrs

Predicted band size: 41 kDa

Multiple bands at 50-65kDa in stimulated or treated cell lysates correspond to different forms of the c-Fos proten.

The single band in red at 37 kDa represents GAPDH protein.



Western blot - Anti-c-Fos antibody [2H2] (ab208942)

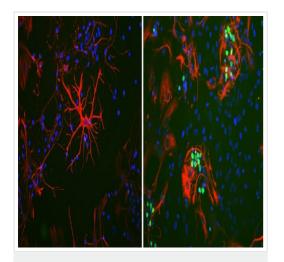
All lanes: Anti-c-Fos antibody [2H2] (ab208942) at 1/1000 dilution

Lane 1: HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate (serum-starved for 36 hours)

Lane 2 : HeLa cell lysate (serum-starved and then stimulated with 20% Fetal Bovine Serum for 2 hours)

Predicted band size: 41 kDa

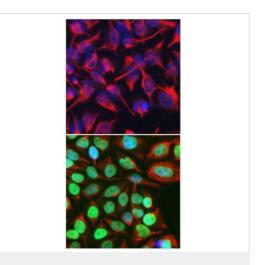
ab208942 recognizes bands in the range of 50-65 kDa, which represent multiple forms of c-Fos. Serum-starvation attenuates c-Fos expression, while 20% FBS strongly stimulates c-Fos expression. Bottom panel: Blot was stripped and probed with a monoclonal antibody against GAPDH, used as loading control.



Immunocytochemistry/ Immunofluorescence - Antic-Fos antibody [2H2] (ab208942)

Rat brain neural cultures (left) and the same cells stimulated with membrane deplorization buffer for 5 hours (right).

This is a salt solution containing 170mM Potassium which depolarizes and stimulates gene expression in neuronal cells but has no effect on glia. Cultures were stained with ab208942 in green and rabbit anti-GFAP in red. Nuclear DNA is revealed in blue with the DNA stain DAPI.



Immunocytochemistry/ Immunofluorescence - Antic-Fos antibody [2H2] (ab208942)

Top panel: HeLa (Human epithelial cell line from cervix adenocarcinoma) cells serum starved for 36 hours and then treated with PBS.

Bottom panel: HeLa cells, treated with 20% FBS for 2 hours following serum–starvation for 36 hours, labeling c-Fos using ab208942 at 1/1000 dilution (green).

Red: Vimentin counterstain. Nuclei labeled with DAPI (Blue).

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