abcam

Product datasheet

# Anti-c-Fos antibody [EPR21930-238] ab222699

Recombinant RabMAb

6 Abreviews 27 References 10 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-c-Fos antibody [EPR21930-238] |
| **Description** | Rabbit monoclonal [EPR21930-238] to c-Fos |
| **Host species** | Rabbit |
| **Specificity** | In IHC-P, no staining is observed on rat tissues with this antibody in our lab. We recommend **ab289723** for work on rat. |
| **Tested applications** | **Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP |
| **Species reactivity** | **Reacts with:** Mouse, Human<br>**Predicted to work with:** Common marmoset |
| **Immunogen** | Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: Jurkat and RAW 264.7 grown in serum free medium overnight, followed by treatment with 200 nM PMA for 4 hours, whole cell lysates. IHC-P: Human bladder carcinoma tissue; Mouse dentate gryus and cerebral cortex tissues. ICC/IF: HeLa cells. Flow: HeLa cells. IP: HeLa grown in serum free medium for 36 hours, followed by addition of 20% FBS for 2 hours whole cell lysate. |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 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** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: PBS, 40% Glycerol, 0.05% BSA |

| | |
|---|---|
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR21930-238 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab222699 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 |
|---|---|---|
| **Flow Cyt (Intra)** | | 1/500. |
| **WB** | | 1/1000. Detects a band of approximately 55-60 kDa (predicted molecular weight: 41 kDa). |
| **IHC-P** | ★★★★☆ (4) | 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **ICC/IF** | ★★★★★ (1) | 1/1000. |
| **IP** | | 1/30. |

## Target

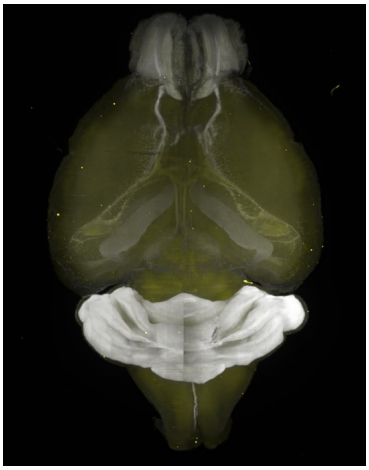
**Function** Nuclear phosphoprotein which forms a tight but non-covalently linked complex with the JUN/AP-1 transcription factor. In the heterodimer, FOS and JUN/AP-1 basic regions each seems to interact with symmetrical DNA half sites. On TGF-beta activation, forms a multimeric SMAD3/SMAD4/JUN/FOS complex at the AP1/SMAD-binding site to regulate TGF-beta-mediated 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.

## Images



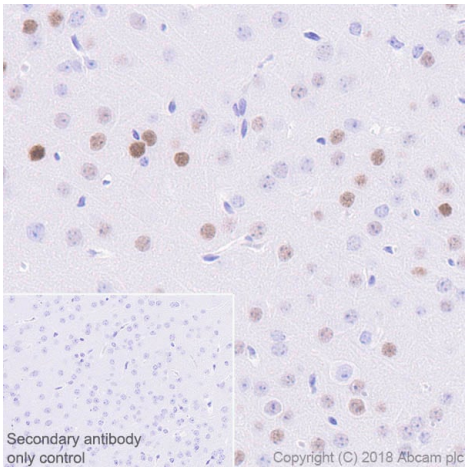
Immunohistochemistry - Anti-c-Fos antibody [EPR21930-238] (ab222699)

Anti-c-FOS antibody ab222699 was used with Tissue clearing kit – CUBIC (**ab316246**) and 3D Tissue Staining Kit – CUBIC (**ab316248**) to penetrate, stain and clear a whole mouse brain. White: nuclear staining, Yellow: c-FOS.

Learn more about **tissue clearing kits, reagents, and protocols** designed to make it easier to stain whole brains and get more data from each valuable tissue sample.

For a whole mouse brain, we recommend starting with 0.6 ug of ab222699 and using a Fab fragment secondary antibody at 0.4 ug to create an antibody complex before 3D staining (see protocol for details). Additive A was used during the staining process.

The sample was imaged using a light-sheet microscope.

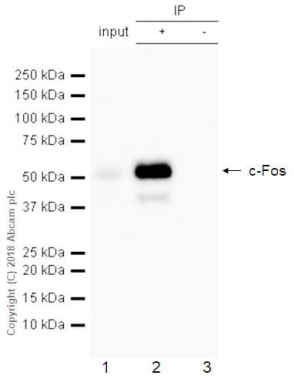



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Fos antibody [EPR21930-238] (ab222699)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling c-Fos with ab222699 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in neurons of mouse cerebral cortex (PMID: 24604295). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat-mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).




Immunoprecipitation - Anti-c-Fos antibody [EPR21930-238] (ab222699)

c-Fos was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) grown in serum free medium for 36 hours, followed by addition of 20% FBS for 2 hours whole cell lysate with ab222699 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab222699 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/5000 dilution.

Lane 1: HeLa grown in serum free medium for 36 hours, followed by addition of 20% FBS for 2 hours whole cell lysate 10 µg (Input).

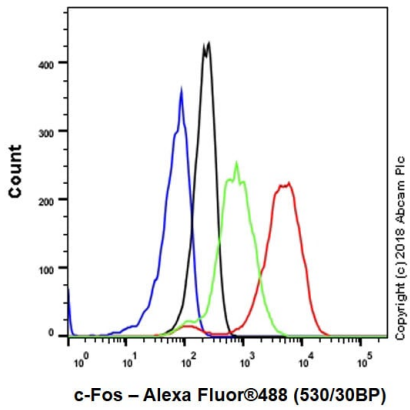
Lane 2: ab222699 IP in HeLa grown in serum free medium for 36 hours, followed by addition of 20% FBS for 2 hours whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab222699 in HeLa grown in serum free medium for 36 hours, followed by addition of 20% FBS for 2 hours whole cell lysate.

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

Exposure time: 30 seconds.

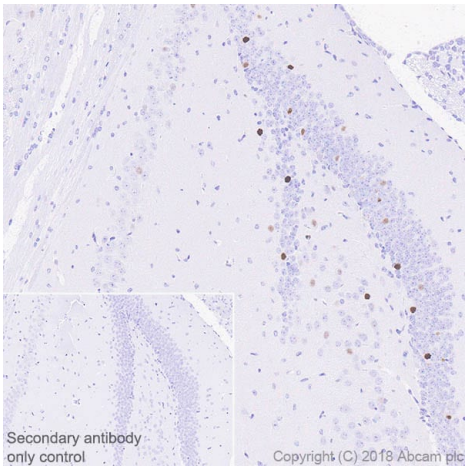
Serum starvation followed by FBS treatment induces the expression of c-Fos (PMID: 14981092).




Flow Cytometry (Intracellular) - Anti-c-Fos antibody [EPR21930-238] (ab222699)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permebalized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line grown in serum free medium for 36 hours, followed by addition of 20% FBS for 2 hours (Red) / Untreated control (Green) labeling c-Fos with **ab22699** at 1/500 dilution compared with a Rabbit monoclonal IgG (**ab172730**) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution.

Serum starvation followed by FBS treatment induces the expression of c-Fos (PMID: 14981092).

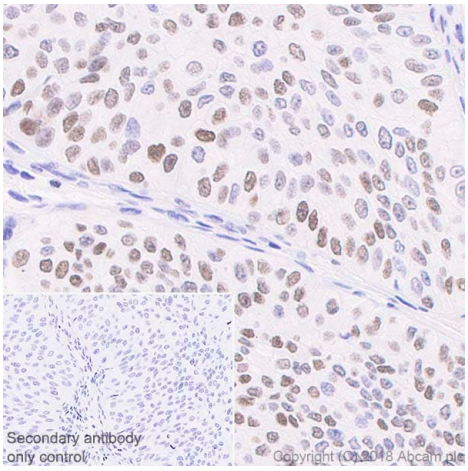



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Fos antibody [EPR21930-238] (ab222699)

Immunohistochemical analysis of paraffin-embedded mouse dentate gyrus tissue labeling c-Fos with ab222699 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Sporadic nuclear staining in mouse dentate gyrus (PMID: 24604295). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat-mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

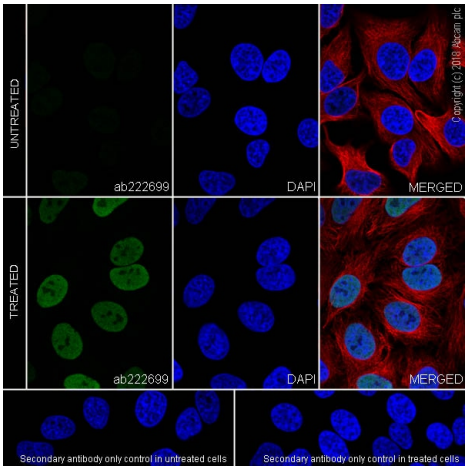



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Fos antibody [EPR21930-238] (ab222699)

Immunohistochemical analysis of paraffin-embedded human bladder carcinoma tissue labeling c-Fos with ab222699 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in tumor cells of human bladder carcinoma (PMID: 28358415). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat-mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

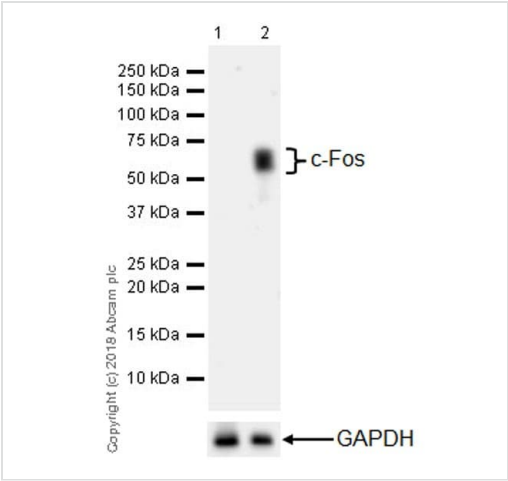



Immunocytochemistry/ Immunofluorescence - Anti-c-Fos antibody [EPR21930-238] (ab222699)

Immunofluorescnt analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeablised HeLa (human cervix adenocarcinoma epithelial cell) cells labeling c-Fos with ab222699 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HeLa cells in serum free medium for 36 hours, followed by addition of 20% fetal bovine serum for 2 hours (PMID: 14981092).

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

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




Western blot - Anti-c-Fos antibody [EPR21930-238] (ab222699)

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/5000 dilution

**Lane 1 :** Untreated RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) grown in serum free medium overnight, whole cell lysate
**Lane 2 :** RAW 264.7 grown in serum free medium overnight, followed by treatment with 200 nM PMA for 4 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

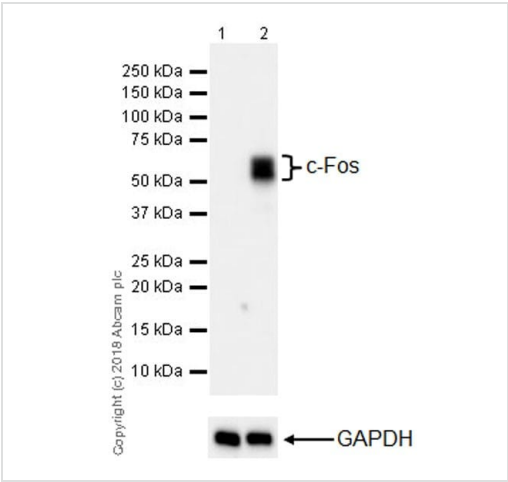
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 41 kDa
**Observed band size:** 55-60 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

PMA treatment induces expression of c-Fos, as documented in the literature (PMID: 24386331, PMID: 23300800, PMID: 25695333).




Western blot - Anti-c-Fos antibody [EPR21930-238] (ab222699)

**All lanes :** Anti-c-Fos antibody [EPR21930-238] (ab222699) at 1/1000 dilution

**Lane 1 :** Untreated Jurkat (human T cell leukemia cell line from peripheral blood) grown in serum free medium overnight, whole cell lysate
**Lane 2 :** Jurkat grown in serum free medium overnight, followed by treatment with 200 nM PMA for 4 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

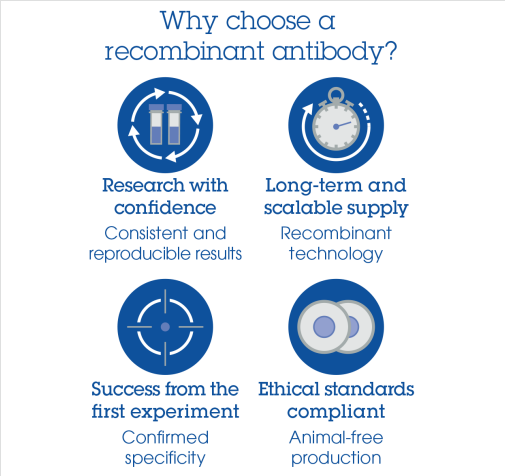
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 41 kDa
**Observed band size:** 55-60 kDa

**Exposure time:** 48 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

PMA treatment induces expression of c-Fos, as documented in the literature (PMID: 24386331, PMID: 23300800, PMID: 25695333).




Anti-c-Fos antibody [EPR21930-238] (ab222699)



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