NEUROMICS

c-Fos Data Sheet

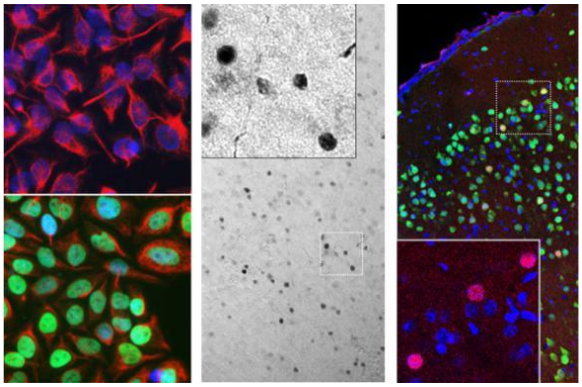
Catalog Number:	MO22162	**Host:**	Mouse
Product Type:	Monoclonal IgG1 Affinity Purified Antibody	**Species Reactivity:**	Human, rat, mouse
Immunogen Sequence:	Full length recombinant human protein expressed in and purified from E. coli HGNC name for this protein is FOS	**Format:**	Liquid, 100 ul aliquot Concentration: 1 mg/ml
Applications:	Immunofluorescence/Immunocytochemistry: 1:1,000 Immunohistochemistry: 1:1,000 Western Blot: 1:1,000-1: 2,000		
Storage:	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. Antibody can also be aliquoted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Avoid repeated freeze-thaw cycles.		

Application Notes

Description/Data:

c-Fos is part of the Fos gene family; which consists of 4 members: FOS, FOSb, FOSL1 and FOSL2. Fos family genes are associated with JUN proteins and other basic leucine ipper (bZIP) domain proteins to create a variety of AP-1 (activator protein-1) complexes. c-Fos is considered to be an immediate-early gene because expression is normally low but rapidly and transiently in response to a wide array of stimuli including serum, growth factors, tumor promoters, cytokines, and UV radiation. It plays an important role in many cellular functions, and is over-expressed in variety of cancers.

Left: MO22162 staining (green) in HeLa cells, which were treated with serum-starvation for 36 hours, followed by 2 hours, 20% FBS stimulation (bottom panel), or PBS treatment (top panel). Green c-Fos staining only localizes in the nuclei of stimulated cells, but not in un-stimulated cells. Cells are counter-stained with our chicken polyclonal antibody against vimentin, CH22108 in red). Blue shows DAPI staining of nucleus. **Middle:** Mouse brain section (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) labeled with MO22162 using a standard HRP-DAB (horseradish peroxidase-3,3'-diaminobenzidine) staining technique. Cells expressing c-Fos show dark color in nucleus. **Right:** Mouse cortical section labeled with MO22162 (red) and our rabbit polyclonal anti Fox3/NeuN (RA22113) antibody (green) using immuno-fluorescent confocal-microscopy. Neurons positive for c-Fos and Fox3/NeuN appear to be yellow. Inset shows an enlarged image of MCA-2H2 staining. Nuclei are labeled with Dapi (blue).

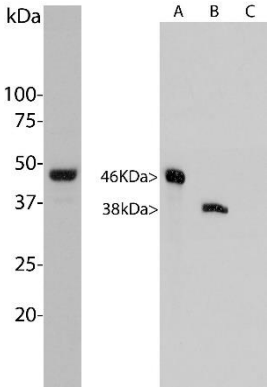


FOR RESEARCH USE ONLY

www.neuromics.com

Neuromics • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail: pshuster@neuromics.com

Top panel: Western blot analysis of c-Fos expression in HeLa cells using MO22162. Lane 1: HeLa cells were serum-starved for 36 hours. 2: Serum-starved HeLa cells were stimulated with 20% FBS (fetal bovine serum) for 2 hours. MO22162 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 our monoclonal antibody against GAPDH, MO25038 used as loading control.



Immunostaining of cells in tissue culture:

The purpose of fixation is denaturing the components of cells enough so that they stay on the dish and can be bound by antibodies, hopefully without destroying cellular morphology. Fixatives such as formalin, paraformaldehyde and glutaraldehyde chemically cross-link proteins, by binding to amino acid side chains, generally the most chemically reactive ones like amines (Lysine, Arginine, Glutamine and Asparagine). This chemical modification can also have the consequence of blocking antibody binding sites. Substances such as acetone and methanol are not true fixatives but are denaturants, which precipitate proteins without covalently modifying them. We routinely use a combination of mild formalin fixation followed by cold methanol for neurons, mixed neuron/glial cultures and most of the widely used human and rodent cell lines. The formalin preserves the cellular morphology quite well, while the methanol further denatures the proteins of the cells and helps keep what is left of the cell adherent to the dish. For soluble proteins it may be necessary to miss the methanol step, but in this case you have to be very careful with the washing steps, as the cells tend to wash off the dish. Certain antibodies may be very sensitive to formalin fixation, so you may have to experiment a little, perhaps missing out that step. The following procedure works for antibodies to most cytoskeletal and signaling molecules. This procedure is good for cells in 6 well tissue culture plates or in 35mm tissue culture dishes. These are just big enough that you can look from above with a typical immunofluorescence microscope through a glass coverslip. This allows you to see the specimens very well and take very high quality pictures. (However note that it's a pain to changes lenses on the microscope if you use the 6 well dishes, since you have to rack the lens right the way up to do this, and you have to take out the two neighboring lenses in the turret since they will hit the other wells of the dish! It's less of a problem with 35mm dishes but still a pain. No procedure is perfect.…).

1. Draw of culture medium with aspirator and add 1 mL of 3.7 % formalin in PBS solution to the dish. (make up from 10 mLs Fisher 37% formalin plus 90mls PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let sit at room temp for 1 minute. (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time round though as it may extract your antigen or help wash your cells off the dish).

2. Take off the formalin/PBS and add 1ml of cold methanol (-20°C, kept in well-sealed bottle in fridge). Let sit for no more than 1 minute.

3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10 µL (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically, 100 µL of hybridoma tissue culture supernatent or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight, exact time not too critical). Can do very gentle shaking for well adherent cell lines (3T3, Hek293 etc.).

4. Remove primary antibody and replace with 1 mL of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

5. Add 0.5 µLs of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit antibodies and are conjugated to ALEXA dyes and were originally marketed by Molecular Probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc. Molecular Probes was bought by Invitrogen, which now markets these reagents). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight). Can do gentle shaking for well adherent cell lines (3T3, HEK293 etc.).

6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

7. Drop on one drop of Fisher mounting medium onto dish and apply 22 mm square coverslip. View in the microscope

FOR RESEARCH USE ONLY

Neuromics • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail: pshuster@neuromics.com