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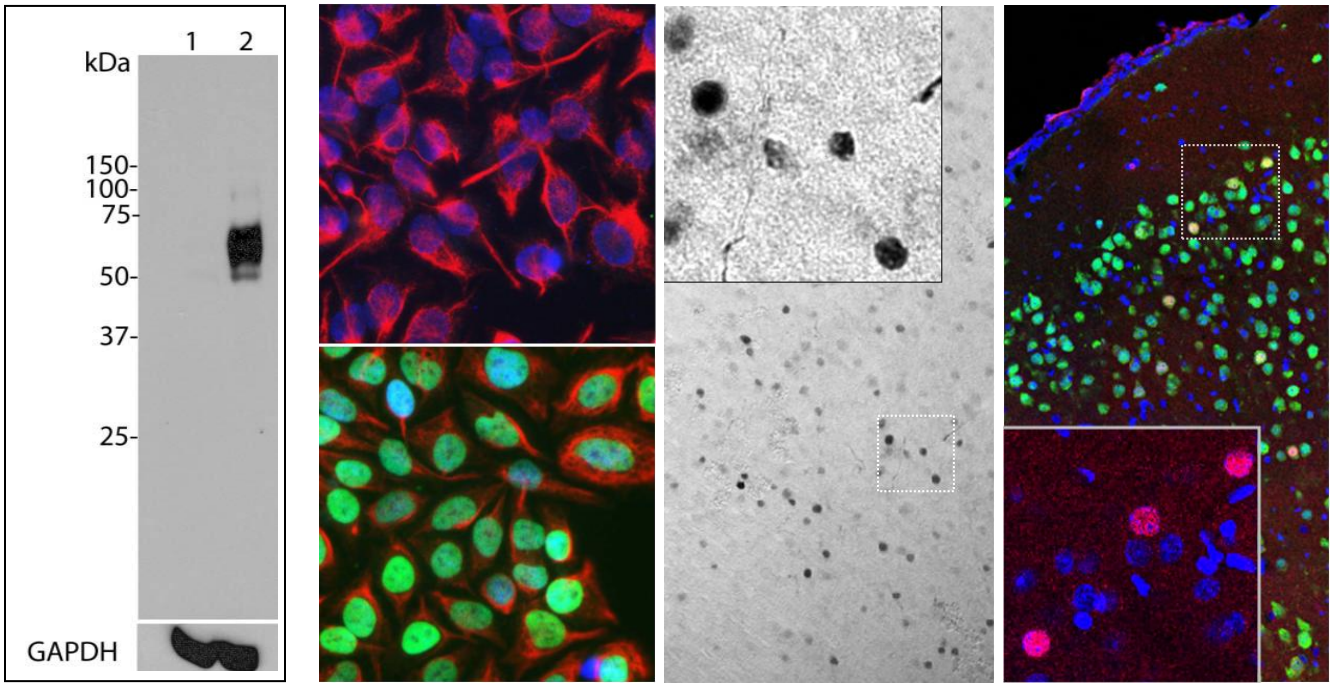
Catalog # MCA-2H2: Mouse Monoclonal Antibody to c-Fos

The Immunogen: The c-Fos is a member of Fos family of transcription factors and is a cellular counterpart of the retroviral oncogene: v-Fos. Other members of Fos family are FosB, Fra-1 and Fra-*2*. Fos proteins are associated with Jun proteins, but also with other basic leucine-zipper (bZIP) proteins to create a variety of AP-1 (activator protein-1) complexes (1). Dimeric complex of AP-1 transcription factor regulate major physiological processes such as cell proliferation, differentiation, neoplastic transformation, apoptosis, and response to stress. c-Fos and c-Jun are the best-studied AP-1 components. They share a number of homologous domains, including adjacent basic leucine zipper motifs, necessary for binding to DNA and for dimerization, respectively. c-Fos and c-Jun-containing AP-1 dimers activate transcription by direct contacts with coactivators, such as the CREB-binding protein (CBP, 3), and constituents of the basal transcription machinery, such as the TATA-binding protein (TBP, 4). c-Fos are considered immediate-early genes because their expression is usually low but inducible rapidly and transiently in response to a wide array of stimuli including serum, growth factors, tumor promoters, cytokines, and UV radiation to allow cells to adapt to environmental changes. c-Fos is subjected to different modifications: phosphorylation activates c-Fos, whereas sumoylation of c-Fos inhibits the AP-1 transcriptional activity (5,6). In the brain, the expression of c-Fos by individual neurons is widely used as a marker of neuronal activity, due to a association of c-Fos expression with electrical stimulation (7,8,9,10). Such work is usually done on histological sections of brain tissue and can be used to trace functional neuroanatomical connections, identify seizure pathways and the sites of action of neuroactive drugs (8). Our MCA-2H2 antibody is proved to be an excellent reagent for these purposes. The MCA-2H2 was generated against full length human recombinant c-Fos protein expressed in and purified from *E. coli.* The HGNC name for this protein is FOS.



Left: Western blot analysis of c-Fos in HeLa cells using MCA-2H2. lane 1: HeLa cells were serum-starved for 36 hours. lane 2: Serum-starved HeLa cells were stimulated with 20% fetal bovine serum for 2 hours. MCA-2H2 recognizes bands in the range of 50-65 kDa, which represent multiple forms of c-Fos. Serum-starvation inhibits, while 20% FBS stimulates c-Fos expression in HeLa cells (top panel). The same blot was stripped and probed with EnCor's monoclonal antibody against GAPDH: **MCA-1D4**, used as loading control (bottom panel). **Middle Left:** MCA-2H2 staining (green) in HeLa cells, which were treated with serum-starvation for 36 hours, followed by 2 hours, 20% FBS stimulation (bottom panel), or followed by PBS treatment (top panel). Green c-Fos staining only localizes in the nuclei of stimulated cells, but not in un-stimulated cells. Counter-stained is EnCor's

chicken polyclonal antibody against vimentin: **CPCA-Vim** (red). Blue shows DAPI staining of nucleus. **Middle Right:** Mouse brain section (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) labeled with MCA-2H2 using a standard HRP-DAB (horsradish peroxidase-3,3'-diaminobenzidine) staining technique. Cells expressing c-Fos show dark color. **Right:** Mouse cortical section labeled with MCA-2H2 (red) and our rabbit polyclonal anti Fox3/NeuN (**RPCA-Fox3**) 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 more images of MCA-2H2 and our rabbit polyclonal antibody to c-Fos: RPCA-c-Fos-AP, go to http://encorbio.com/Data/cFosabpics.html.

Antibody characteristics: MCA-2H2 is a mouse IgG1 class antibody with a κ light chain. The antibody solution is purified from tissue culture supernatant and is at a concentration of 1 mg/mL in phosphate buffered saline. The antibody recognizes c-Fos specifically both in western blots and in immunofluorescence experiments. On blots, MCA-2H2 reveals bands that appear in the range of 50-65 kDa, and on cells in tissue culture the antibody stains the nuclei of activated cells. The antibody is known to work on human and rodent cells and, since c-Fos is highly conserved, it is likely that the antibody is effective on other species also.

Suggestions for use: The antibody solution can be used at dilutions of at least 1:1,000 in immunofluorescence experiments. In western blotting using chemiluminescence it can be used at dilutions of 1:2,000. Antibody preparation contains 5 mM sodium azide preservative (Link to http://www.encorbio.com/MSDS/Azide.pdf for Material Safety Data Sheet).

Storage Instructions: Shipped on ice. Please store at 4°C for regular uses. For long term storage, please leave frozen at -20°C and avoid freeze/thaw cycles.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis

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