Anti-c-Fos Antibody [PSH05-77]

HA722666

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IHC-P, IF-Cell, IHC-Fr, mIHC, IF-Tissue
Molecular Wt:	Predicted band size: 41 kDa
Clone number:	PSH05-77

Description: Protein c-Fos is a proto-oncogene that is the human homolog of the retroviral oncogene v-fos. It is encoded in humans by the FOS gene. It was first discovered in rat fibroblasts as the transforming gene of the FBJ MSV (Finkel–Biskis–Jinkins murine osteogenic sarcoma virus). It is a part of a bigger Fos family of transcription factors which includes c-Fos, FosB, Fra-1 and Fra-2. It has been mapped to chromosome region 14q21→q31. c-Fos encodes a 62 kDa protein, which forms heterodimer with c-jun (part of Jun family of transcription factors), resulting in the formation of AP-1 (Activator Protein-1) complex which binds DNA at AP-1 specific sites at the promoter and enhancer regions of target genes and converts extracellular signals into changes of gene expression. It plays an important role in many cellular functions and has been found to be overexpressed in a variety of cancers.

Immunogen: Recombinant protein within human Protein c-Fos aa 1-380.

Positive control: HeLa serum starved for 40 hours then add 20% FBS for 2 hours cell lysate, RAW264.7 serum starved for 16 hours then add 200nM PMA for 4 hours cell lysate, C6 serum starved for 16 hours then add 10% FBS for 30 minutes cell lysate, human colon cancer tissue, mouse brain tissue, mouse hippocampus tissue, rat cerebellum tissue, mouse cerebrum tissue.

Subcellular location: Nucleus, Endoplasmic reticulum, Cytoplasm, cytosol.

Database links: SwissProt: P01100 Human | P01101 Mouse | P12841 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:500-1:1,000
IF-Cell	1:100
IHC-Fr	1:500-1:4,000 (mouse), 1: 200-1:500 (rat)
mIHC	1:200-1:500
IF-Tissue	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

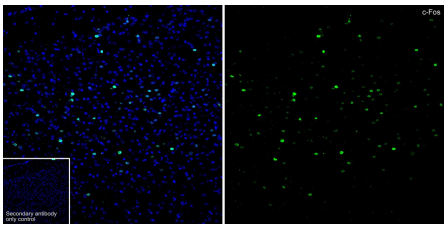

Fig1: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex (restraint stress induced)

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

Important Notice: Blocking buffer and antibody dilution buffer are recommended to use **TBS buffer** instead of PBS buffer.

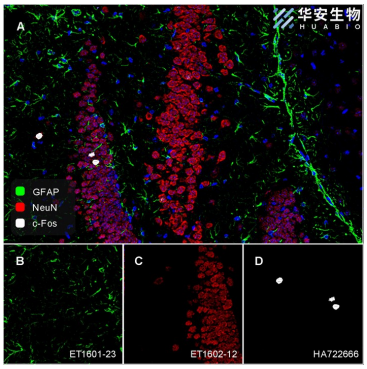

Fig2: Fluorescence multiplex immunohistochemical analysis of mouse hippocampus (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-GFAP (ET1601-23, Green), anti-NeuN (ET1602-12, Red) and anti-c-Fos (HA722666, White) on hippocampus. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1601-23 (1/1,000 dilution), ET1602-12 (1/1,000 dilution) and HA722666 (1/200 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

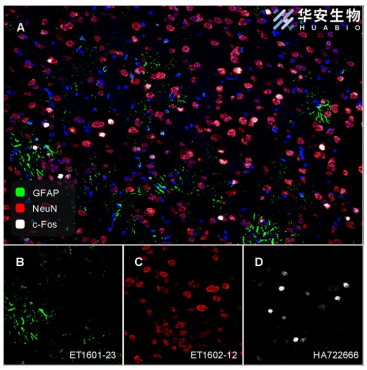

Fig3: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-GFAP (ET1601-23, Green), anti-NeuN (ET1602-12, Red) and anti-c-Fos (HA722666, White) on brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1601-23 (1/1,000 dilution), ET1602-12 (1/1,000 dilution) and HA722666 (1/200 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

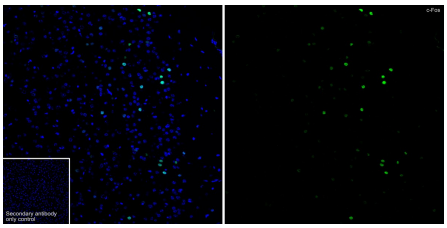

Fig4: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/1,000

Antigen retrieval: Not required

Important Notice: Blocking buffer and antibody dilution buffer are recommended to use **TBS buffer** instead of PBS buffer.

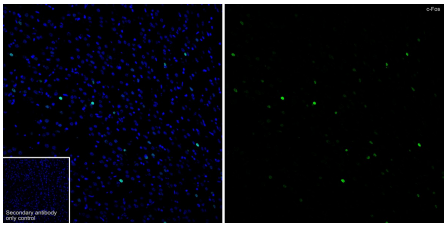

Fig5: Application: IHC-Fr

Species: Rat

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: Not required

Important Notice: Blocking buffer and antibody dilution buffer are recommended to use **TBS buffer** instead of PBS buffer.

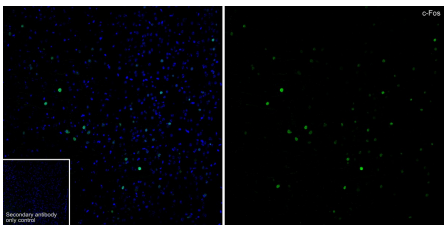

Fig6: Application: IF-tissue

Species: Mouse

Site: Cerebral cortex (restraint stress induced)

Sample: Paraffin-embedded section

Antibody concentration: 1/1,000

Important Notice: Blocking buffer and antibody dilution buffer are recommended to use **TBS buffer** instead of PBS buffer.

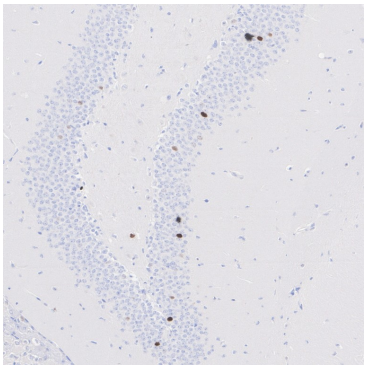

Fig7: Immunohistochemical analysis of paraffin-embedded restraint stress induced mouse hippocampus tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

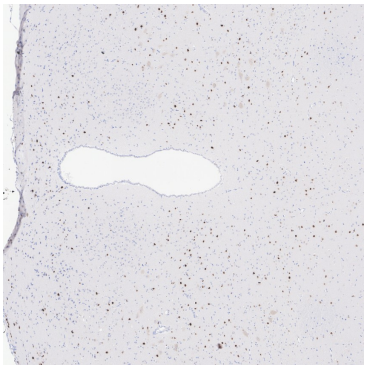

Fig8: Immunohistochemical analysis of paraffin-embedded restraint stress induced mouse brain tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

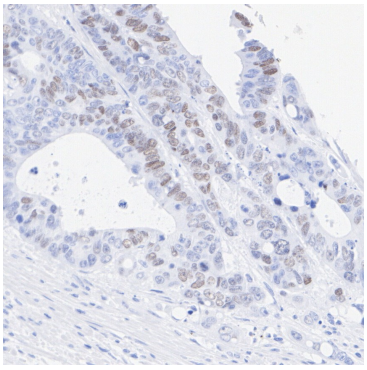

Fig9: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

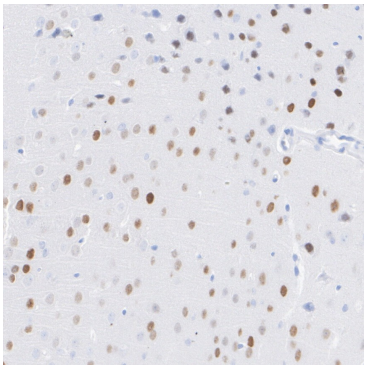

Fig10: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

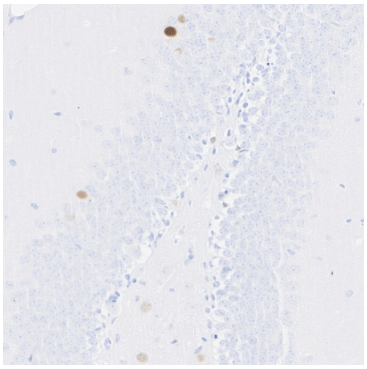

Fig11: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

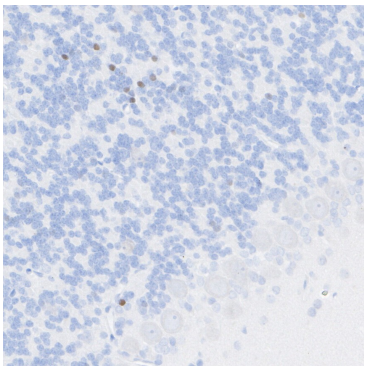

Fig12: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/800 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

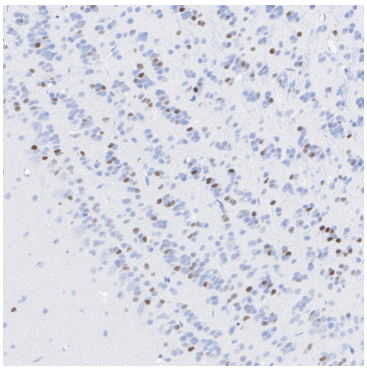

Fig13: Immunohistochemical analysis of paraffin-embedded rat brain (olfactory bulb) tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

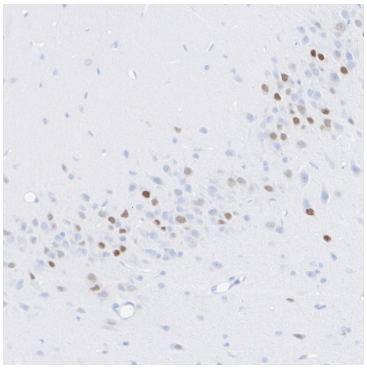

Fig14: Immunohistochemical analysis of paraffin-embedded rat brain (piriform area) tissue with Rabbit anti-c-Fos antibody (HA722666) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722666) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

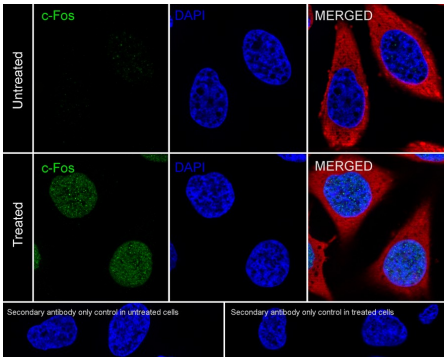

Fig15: Immunocytochemistry analysis of HeLa cells serum starved for 40 hours then add 20% FBS for 2 hours labeling c-Fos with Rabbit anti-c-Fos antibody (HA722666) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-c-Fos antibody (HA722666) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

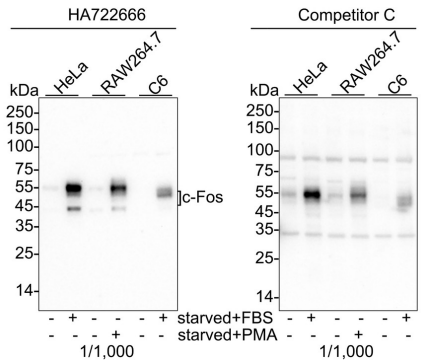

Fig16: Western blot analysis of c-Fos on different lysates with Rabbit anti-c-Fos antibody (HA722666) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa serum starved for 40 hours then add 20% FBS for 2 hours cell lysate
Lane 3: RAW264.7 cell lysate
Lane 4: RAW264.7 serum starved for 16 hours then add 200nM PMA for 4 hours cell lysate
Lane 5: C6 cell lysate
Lane 6: C6 serum starved for 16 hours then add 10% FBS for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: Lane 1-6 (left): 3 minutes; ECL: K1801;
Exposure time: Lane 1-6 (right): 1 minute 2 seconds; ECL: K1802;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722666) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

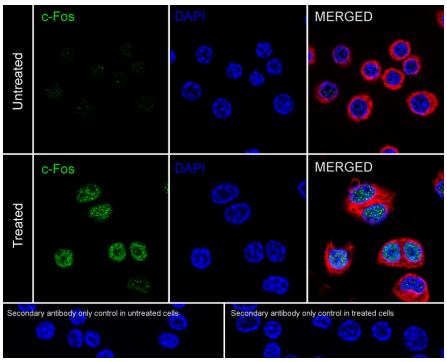

Fig17: Immunocytochemistry analysis of RAW264.7 cells serum starved for 16 hours then add 200nM PMA for 4 hours labeling c-Fos with Rabbit anti-c-Fos antibody (HA722666) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-c-Fos antibody (HA722666) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

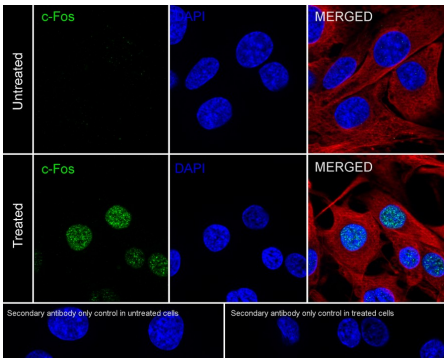

Fig18: Immunocytochemistry analysis of C6 cells serum starved for 16 hours then add 10% FBS for 30 minutes labeling c-Fos with Rabbit anti-c-Fos antibody (HA722666) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-c-Fos antibody (HA722666) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Matsuoka K et al. Metabolic rewiring controlled by c-Fos governs cartilage integrity in osteoarthritis. Ann Rheum Dis. 2023 Sep
2. Osada N et al. c-FOS is an integral component of the IKZF1 transactivator complex and mediates lenalidomide resistance in multiple myeloma. Clin Transl Med. 2023 Aug