For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Phosphorylated GFAP (Ser8)

Code No.	Clone	Subclass	Quantity	Concentration
MY-01-3	YC10	Mouse IgG1	50 µg	1 mg/mL

BACKGROUND: Intermediate filaments (IFs) constitute major components of the cytoskeleton and the nuclear envelope in most cell types. Unlike other cytoskeletons such as microtubules and actin filaments, the protein components of IFs vary in a cell-, tissue-, and differentiation-dependent manner. Although IFs were thought to be relatively stable compared with actin filaments and microtubules, intensive in vitro investigations revealed that site-specific phosphorylation by several kinases, such as protein kinase A (PKA), protein kinase C (PKC), Ca^{2+}/Calmodulin kinase II (CaMKII), cdc2 kinase, Aurora-B, and Rho-kinase alters dynamically their structure and induces filament disruption. Glial fibrillary acidic protein (GFAP) belongs to IF proteins. GFAP is a useful marker of mature astrocytes and mutation in the GFAP gene has recently been associated with Alexander disease. Inagaki et al. reported that Cdc2 kinase phosphorylates GFAP at Ser8, which appeared at G2-M phase transition in the entire cytoplasm. Rho-kinase phosphorylates GFAP at Ser13 and Ser34 but not at Ser8.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone YC10) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with KLH conjugated RRRVTpSAARRS corresponding to GFAP (3-13 aa).

FORMULATION: 50 µg IgG in 50 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody recognizes the local phosphorylation site sequence (TpSAARR, 7-12 aa) specifically, and does not react with unphosphorylated GFAP. This antibody does not react with another phosphorylated intermediate filament proteins (Vimentin, Desmin, Keratin No.8, No.18 and Neurofilament 68K, 160K, 200K).

INTENDED USE:
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APPLICATIONS:
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

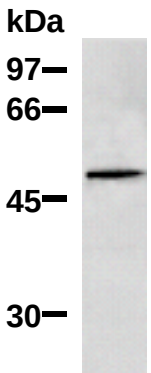
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	U251		
Reactivity on WB	+	-	-

*Reactivity of YC10 to other species is not confirmed in our laboratory. However, it is reported that this clone cross-reacts with porcine and bovine phosphorylated GFAP in the reference number 8).

REFERENCES:
1) Kawajiri, A., *et al., Mol. Biol. Cell* **14**, 1489-1500 (2003)
2) Takemura, M., *et al., Genes Cells* **7**, 295-307 (2002)
3) Takemura, M., *et al., J. Neurosci.* **22**, 6972-6979 (2002)
4) Nagata, K., *et al., Genes Cells* **6**, 653-664 (2001)
5) Yasui, Y., *et al., J. Cell Biol.* **143**, 1249-1258 (1998)
6) Kosako, H., *et al., J. Biol. Chem.* **272**, 10333-10336 (1997)
7) Matsuoka, Y., *et al., EMBO J.* **11**, 2895-2902 (1992)
8) Yano, T., *et al., BBRC* **175**, 1144-1151 (1991)

Clone YC10 is used in these references.



Western blot analysis of phospho GFAP (Ser 8) expression in U251 using MY-01-3.

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PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; U251)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$ cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.
4) The glass slide was washed with PBS 3 times.
5) Permeabilize the cells by immersing the slide in methanol for 10 minutes at -20°C.
6) The glass slide was washed with PBS 3 times.
7) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add 100 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add Permafluor™ aqueous mounting medium (MBL; code no. IM-0752) onto the slide, then put a cover slip on it.

RELATED PRODUCTS:

D098-3	Anti-Phosphorylated GFAP (Thr7) (TMG7)
D121-3	Anti-Phosphorylated GFAP (Ser13) (KT13)
D076-3	Anti-Phosphorylated Vimentin (Ser55) (4A4)
D093-3	Anti-Phosphorylated Vimentin (Ser71) (TM71)
D094-3	Anti-Phosphorylated Vimentin (Ser38) (TM38)
D095-3	Anti-Phosphorylated Vimentin (Ser82) (MO82)
D096-3	Anti-Phosphorylated Vimentin (Ser6) (MO6)
D099-3	Anti-Phosphorylated Vimentin (Ser33) (YT33)
D122-3	Anti-Phosphorylated Vimentin (Ser50) (TM50)
PD005	Anti-Vimentin Fragment (V1) (polyclonal)