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

MONOCLONAL ANTIBODY

Anti-Phosphorylated GFAP (Ser13)

Code No.	Clone	Subclass	Quantity	Concentration
D121-3	KT13	Mouse IgG1	100 µL	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, 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 GFAP was phosphorylated specifically at Thr-7, Ser-13, and Ser-34 by Rho-kinase and Aurora-B and all of these sites were phosphorylated at the cleavage furrow during cytokinesis.

SOURCE: This antibody was purified from ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with the KLH-pG13 (CSAARRpSYVSSL) peptide.

FORMULATION: 100 µg IgG in 100 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 reacts with phosphorylated GFAP (Ser13) (50 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 10 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 20 µg/mL
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	U251	Not Tested	Not Tested
Reactivity on WB	+		

INTENDED USE:

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3) Takemura, M., *et al., Genes Cells* **7**, 295-307 (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) Inagaki, M., *et al., J. Biochem*. **121**, 407-414 (1997)
8) Inagaki, M., *et al., BioEssays*. **18**, 481-487 (1996)
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10) Inagaki, N., *et al., Trends Biochem. Sci*. **19**, 448-452 (1994)
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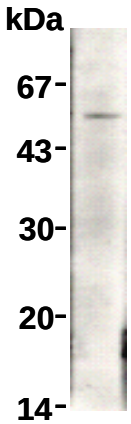
Clone KT13 is used in reference number 1)-9) and 11).



Western blot analysis of phosphorylated GFAP (Ser13) in U251 cells using D121-3.

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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 of U251 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in PBS containing 3.7% Formaldehyde for 10 minutes on ice.
3) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
4) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 2 hours at 37 °C (Optimization of antibody concentration or incubation condition are recommended if necessary.)
5) Prepare a wash container such as a 500 mL beaker with a stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another washes once more.
6) Add 100 µL of 1:160 FITC conjugated anti-mouse IgG (MBL; code no. 238) onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
7) Wash the slide in a plenty of PBS as in the step 5).
8) Add 1 µg/mL of Propidium Iodide (PI) onto the cells. Incubate for 15 minutes at room temperature.
9) Wash the slide in a plenty of PBS as in the step 5).
10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add Permafluor™ aqueous mounting medium (MBL; code no. IM-0752) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; U251)

RELATED PRODUCTS:

D097-3	Anti-GFAP (MO389)
D098-3	Anti-Phosphorylated GFAP (Thr7) (TMG7)
MY-01-3	Anti-Phosphorylated GFAP (Ser8) (YC10)
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)