

ORDERING INFORMATION

Catalog Number: MAB5595

Clone: 528322

Lot Number: CCGO01

Size: 100 μ g

Formulation: 0.2 μ m filtered solution in PBS with 5% trehalose

Storage: -20° C

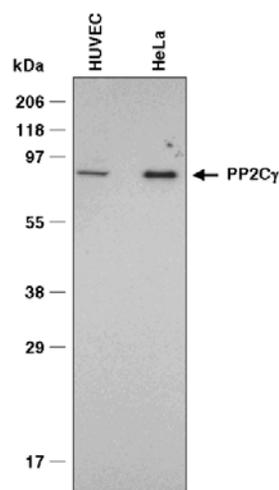
Specificity: human PP2C γ

Immunogen: *E. coli*-derived rhPP2C γ

Ig class: mouse IgG₁

Recommended Applications:

Western blot
Immunohistochemistry



Detection of PP2C γ with MAB5595.

Lysates from human HUVEC and HeLa cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 0.25 μ g/mL anti-PP2C γ , as described in *Protocols for Immunoblotting*.

Background

Protein phosphatase 2C isoform gamma (PP2C gamma; also PPM1G) is a widely expressed, 75 kDa member of the PP2C family of Ser/Thr protein phosphatases. Human PP2C gamma is 546 amino acids (aa) in length. The apparent mass is much larger than the predicted mass of 59 kDa because of the presence of an acidic region (aa 259 - 326) rich in glutamate and aspartate residues, causing the protein to move anomalously on SDS-PAGE. The protein contains numerous sites for phosphorylation by casein kinase II, particularly within the acidic domain.

Preparation

The antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived full length recombinant human PP2C γ (rhPP2C γ ; aa 1 - 546; Accession # O15355). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 μ m filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human PP2C γ at 75 kDa using Western blots.

Applications

Western blot - An antibody concentration of 0.25 μ g/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution

2% nonfat dry milk in
Blotting Buffer
Adjust pH to 7.4

Antibody Solution

2% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.25 μ g/mL mouse anti-PP2C γ .
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings

- To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2×10^5 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.

Immunohistochemistry - This antibody was used at a concentration of 25 μ g/mL with appropriate secondary reagents to detect PP2C γ in hepatocytes of paraffin-embedded normal and cancer human liver tissue sections. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

Optimal dilutions should be determined by each laboratory for each application.

For immunohistochemistry images, please refer to our website at <http://www.RnDSystems.com/go/ihc>.