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AM26569AF-N Monoclonal Antibody to 14-3-3 protein gamma (1-12) - Azide Free

Alternate names:	KCIP-1, Protein kinase C inhibitor protein 1, YWHAG
Quantity:	0.1 mg
Concentration:	1.0 mg/ml
Background:	The 14-3-3 protein family comprises critical regulatory molecules involved in signaling during cell division, proliferation, and apoptosis. 14-3-3 γ, a subtype of the 14-3-3 family of proteins, was thought to be brain- and neuron-specific. However, 14-3-3 γ was not neuron-specific but also expressed in astrocytes. Endogenous 14-3-3 γ proteins in immature astrocytes appeared filamentous and co-localized with filamentous actin (F-actin). And, 14-3-3 γ proteins play a role in cytoskeletal function during the process of cell division and apoptosis in astrocytes in association with actin. This expression is induced in arterial trauma by cytokines, and suggests that this protein may play an important role in progression of vascular proliferative diseases.
Uniprot ID:	P61981
NCBI:	NP_036611.2
GeneID:	7532
Host / Isotype:	Mouse / IgG2a
Clone:	KC21
Immunogen:	KLH-conjugated human 14-3-3γ N-terminal peptides (1-12 aa)
Format:	**State:** Liquid Ig fraction **Purification:** Protein A agarose **Buffer System:** PBS containing 50% glycerol, pH 7.2. No preservative is contained.
Applications:	Western blot: 0.1 µg/ml for chemiluminescence detection system. Immunoprecipitation: 2 µg/100 µg of cell extract from $5x10^6$ cells. For details see protocol below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Molecular Weight:	33 kDa
Specificity:	This antibody reacts with 14-3-3γ, which the N-terminal Met is removed as a result of acetylated the N-terminal Val, on Western blotting. This antibody does not recognize unprocessed (non-modified) 14-3-3γ.
Species Reactivity:	**Tested:** Human, Mouse, Rat
Add. Information:	This product was originally produced by MBL International.
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing. Shelf life: One year from despatch.

General Readings:

1. Chen, X.Q., et al., Biochem. Biophys. Res. Commun. 296, 657-663 (2002).

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 4oC 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 4oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the 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 2 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/cm2 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 4oC.
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 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; A431, K562, NIH/3T3, mouse brain, PC12, rat brain)

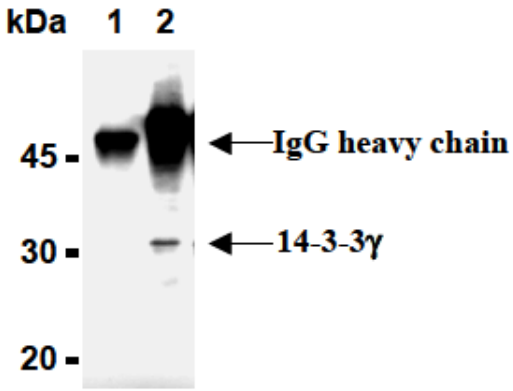
Immunoprecipitation

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 4oC 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 4oC and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the APPLICATIONS into 100 µg of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4oC.
4) Add 20 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4oC.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes,

and centrifuge for 5 minutes. Load 10 µL of the sample per lane in a 1mm thick SDS-polyacrylamide gel for electrophoresis.
7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 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.
8) 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 4oC.
9) 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.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
11) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (gamma) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
12) Wash the membrane with PBS-T (5 minutes x 6 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive control for Immunoprecipitation; A431)

Pictures:

Immunoprecipitation of 14-3-3γ from A431 cells with mouse IgG2a (1) or AM26569AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26569AF-N.



Western blot analysis of 14-3-3γ expression in A431 (1), K562 (2), NIH/3T3 (3), mouse brain (4), PC12 (5) and Rat brain (6) using AM26569AF.