

Agarose-Conjugated Mouse Monoclonal Anti-Phospho-Tyrosine Antibody

ORDERING INFORMATION

Catalog Number: AGM1676

Clone: 179003

Lot Number: JYV02

Size: 250 μ L beads in 500 μ L suspension

Storage: 2° - 8° C

Specificity: proteins containing
Phospho-Tyrosine

Immunogen: Phospho-Tyrosine:KLH

Ig Type: mouse IgG₁

Application: Immunoprecipitation

Preparation

This antibody was produced using a hybridoma elicited from a mouse immunized with short peptides containing Phospho-Tyrosine coupled to KLH. The IgG fraction of the hybridoma culture supernatant was purified by protein G chromatography and conjugated with agarose.

Formulation

Phosphate-buffered saline (PBS) with preservative.

Storage

Upon receipt, centrifuge tube to pellet agarose that may have collected in the cap during shipment. The agarose should be stored at 2° - 8° C. **Do not freeze.**

Specificity

The antibody detects endogenous proteins containing phosphorylated Tyrosine residues. ELISA and 2D Western blot analyses using ligand- and pervanadate-treated cell lysates indicate that clone 179003 binds Phospho-Tyrosine in a broad manner largely independent of the surrounding amino acid sequence. The antibody does not cross-react with proteins or peptides containing phosphorylated serine or threonine residues.

Application

For immunoprecipitation, use 10 μ L of beads (20 μ L of agarose slurry) per 0.2 - 0.5 mL of total cell lysate.

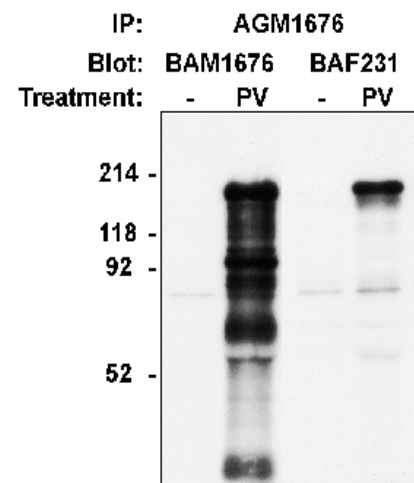
The optimal amount of agarose-conjugated antibody that will quantitatively immunoprecipitate the protein of interest should be empirically determined for each cell model.

Protocol for Immunoprecipitation

Wash Buffer	Cell Lysis Buffer	2x SDS Gel Sample Buffer
20 mM Tris, pH 8.0	Wash Buffer	250 mM Tris, pH 6.8
0.15 M NaCl	containing:	20 mM dithiothreitol
2 mM EDTA	0.25 mM PMSF	6% SDS
1% NP-40	1 μ g/mL aprotinin	10% glycerol
10% glycerol	1 μ g/mL chymostatin	10 mM NaF
1 mM Na ₃ VO ₄	1 μ g/mL leupeptin	bromophenyl blue

Cell lysis: Cells are rinsed three times with cold PBS and cell protein is extracted by solubilization of 1×10^6 - 5×10^6 cells per mL of cold Cell Lysis Buffer. The extraction mixtures are rocked at 4° C for 30 - 60 minutes. Cell lysates are then centrifuged at 3,000 x g for 5 minutes to remove insoluble material.

Immunoprecipitation: 20 μ L of agarose-conjugated mouse anti-Phospho-Tyrosine antibody slurry is added to 200 μ L of cell lysate and the mixture is rocked for 16 hours at 2° - 8° C. The agarose-absorbed complexes are centrifuged for 0.5 minutes in a microcentrifuge, resuspended in Wash Buffer by trituration with a glass Pasteur pipette, and then repelleted. The complexes are washed a total of three times with Wash Buffer, suspended in PBS, and transferred to a new tube before the final centrifugation. The washed pellet is suspended in 25 - 50 μ L of 2x SDS Gel Sample Buffer by vortexing and then incubated for 3 minutes in a boiling water bath. The agarose is then pelleted, the supernatant is resolved by SDS-PAGE, proteins are transferred to Immobilon-P membrane (Millipore), and the membrane immunoblotted with the appropriate antibodies.



Detection of Tyrosine-phosphorylated proteins with AGM1676. Lysates from A431 cells either untreated (-) or treated with 300 mM pervanadate (PV) were incubated with agarose-conjugated mouse anti-Phospho-Tyrosine, as described in *Protocols for Immunoprecipitation*. Immunoblotting antibodies were either biotinylated anti-Phospho-Tyrosine (R&D Systems, BAM1676) or biotinylated anti-EGFR (R&D Systems, BAF231), followed by Streptavidin-HRP (R&D Systems, DY998) and ECL detection.

Transfer electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.

1. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 µg/mL mouse anti-Phospho-Tyrosine.
2. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
3. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems, Catalog # HAF007).
4. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
5. Detect with Chemiluminescent detection reagent.

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