

Product Information

ANTI-FLAG® antibody, Rat monoclonal clone 6F7, purified from hybridoma cell culture

Catalog Number **SAB4200071**

Product Description

ANTI-FLAG antibody, Rat monoclonal (rat IgG1 isotype) is derived from the hybridoma 6F7 produced by the fusion of mouse myeloma cells and splenocytes from rat immunized with the FLAG peptide. The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

ANTI-FLAG antibody, Rat monoclonal recognizes N-terminal, C-terminal and internal Flag-tagged fusion proteins. The product is especially recommended for identifying C-terminal FLAG-tagged fusion proteins. The antibody may be used in various immunochemical techniques including immunoblotting and immunoprecipitation.

Epitope tags provide a method to localize gene products in a variety of cell types, study the topology of proteins and protein complexes, identify associated proteins, and characterize newly identified, low abundance, or poorly immunogenic proteins when protein specific antibodies are not available. Tagging with the FLAG peptide sequence may be done at the N-terminus, N-terminus preceded by a methionine residue, C-terminus, or at internal positions of the target protein. FLAG may also be placed in association with other tags.¹ The small size of the FLAG tag or sequence and its high hydrophilicity tend to decrease the possibility of interference with the protein expression, proteolytic maturation, antigenicity, and function.

The N-terminal FLAG peptide sequence contains a unique enterokinase cleavage site allowing it to be completely removed from the purified fusion proteins. Cleavage of the C-terminal FLAG peptide from a fusion protein catalyzed by Cu²⁺ ions has been reported.² A sequence motif with five out of eight amino acid residues identical to the FLAG peptide is found in both rat and mouse Mg²⁺ dependent protein β -phosphatase,³ as well as in the human and bovine enzyme.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: ~ 1.0 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze at -20 °C in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working antibody concentration of 0.5-1.0 μ g/ml is recommended using extracts of transiently transfected cells expressing C-terminal-FLAG- tagged protein.

Immunoprecipitation: a working antibody amount of 2.5-5.0 μ g is recommended using lysates of transiently transfected cells expressing C-terminal-FLAG-tagged protein.

Note: In order to obtain the best results and assay sensitivity with various techniques and preparations, we recommend determining optimal working dilutions by titration.

Procedures

Procedure for Immunoblotting

1. Separate FLAG-tagged fusion proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5–20 µg of total lysate protein per lane.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in Dulbecco's phosphate buffered saline (PBS, Cat. No. D8537) at room temperature for 1 hour.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN[®] 20 (PBS-TWEEN 20, Cat. No. P3563) at room temperature.
5. Incubate the membrane with Anti-FLAG antibody as the primary antibody using an optimized concentration in PBS containing 0.5% NFDM at room temperature with agitation for 2 hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20 at room temperature.
7. Incubate the membrane with Anti-Rat IgG-Peroxidase, Cat. No. A9542, as the secondary antibody at the recommended concentration in PBS containing 0.05% TWEEN 20. Incubate at room temperature for 1 hour. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20 at room temperature.
9. Treat the membrane with a peroxidase substrate.

Note: Using less Anti-FLAG antibody may help to reduce background and cross-reactivity.

Procedure for Immunoprecipitation

Note: The amount of cell lysate to be used for immunoprecipitation depends on the level of expression of the tagged protein and the specific application.

1. To 0.1 to 1.0 mL of a cell lysate containing FLAG-tagged protein add Anti-FLAG antibody and incubate on a rotator for 2 hours to overnight at 4 °C (see Note above).
2. Centrifuge 20-40 µL Protein G-agarose beads, Cat. No. P3296, for 1 min 12,000 x g, and then wash twice with 1 ml RIPA buffer (50 mM Tris Base, 0.25 % w/v Deoxycholate, 1% IGEPAL[®], 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4 °C.
3. Add the mixture from step 1 to the beads and incubate on a rotator for 2 hours at 4 °C.
4. Spin down beads; remove supernatant.
5. Wash beads four times with 1 ml RIPA buffer and one time with PBS, Cat. No. D8537, by vortex and short spin.
6. Resuspend pellet in 30 µL 2X SDS -PAGE sample buffer. Boil sample for 5 minutes and spin down. The sample is ready to be loaded on a SDS-PAGE gel.

References

1. Robeva, A.S., et al., *Biochem. Pharmacol.*, **51**, 545-555 (1996).
2. Humphreys, D.P., et al., *Protein Eng.*, **12**, 179-184 (1999).
3. Schafer, K., and Braun, T., *Biochem. Biophys. Res. Commun.*, **207**, 708-714 (1995).

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