

Product Information

Monoclonal ANTI-FLAG®, clone SIG1-25

produced in rabbit, ascites fluid

Catalog Number **F2555**

Product Description

Monoclonal ANTI-FLAG (Rabbit IgG) is a rabbit monoclonal derived from the hybridoma SIG1-25 produced by the fusion of rabbit myeloma cells and splenocytes from rabbits immunized with the FLAG peptide sequence conjugated to KLH.

Monoclonal ANTI-FLAG reacts with N-terminal FLAG® fusion proteins. The product is useful in ELISA, immunoblotting, and immunofluorescence staining. In immunoblotting assays the antibody can detect 5 ng per lane of N-terminal FLAG-BAP fusion protein, Catalog Number P7582.

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 catalyzed by Cu²⁺ ions of the C-terminal FLAG peptide from a fusion protein 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

The product is provided as ascites fluid containing 15 mM sodium azide as a preservative.

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 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 dilution of 1:1,000-1:2,000 is recommended using extracts of transiently transfected cells expressing FLAG-tagged protein.

Immunocytochemistry: a working dilution of 1:125-1:250 is recommended using transiently transfected cells expressing FLAG-tagged proteins.

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

Procedure

Procedure for Immunoblotting

1. Separate FLAG-tagged fusion proteins from sample lysates using a standard SDS-PAGE protocol. Load 2.5–20 μ g 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 (NFD) in phosphate buffered saline (PBS, Catalog Number D8537) at room temperature for 1 hour.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563, at room temperature.

5. Incubate the membrane with ANTI-FLAG antibody as the primary antibody using an optimized concentration in PBS containing 1% 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-Rabbit IgG (whole molecule)–Peroxidase, Catalog Number A0545, 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 Indirect Immunofluorescent Staining of Cultured Cells

1. Grow transfected cultured cells expressing the FLAG tagged fusion protein of choice on sterile coverslips or slides at 37 °C.
2. Wash the cells briefly in PBS.
3. Fix the cells in cooled methanol for 10 minutes at –20°C and then in cooled acetone for 1 minute at –20 °C.
4. Wash the fixed cells twice in PBS (5 minutes each wash).
Note: Blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5, may minimize non-specific adsorption of the antibodies.

5. Incubate the fixed cells cell-side-up with ANTI-FLAG antibody as the primary antibody using an optimized concentration in PBS. Incubate at room temperature for 1 hour.
6. Wash the fixed cells three times in PBS (5 minutes each wash).
7. Incubate the fixed cells cell-side-up with Anti-Rabbit IgG (whole molecule)–FITC, Catalog Number F9887, as the secondary antibody at the recommended concentration in PBS containing 1% NFDM. Incubate at room temperature for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Cover the cells using a coverslip with aqueous mounting medium and examine using a fluorescence microscope with appropriate filters.

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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