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Product Information

Monoclonal Anti-FLAG®-Peroxidase
Clone 6F7
produced in rat, purified immunoglobulin

Catalog Number **SAB4200119**

Product Description

Monoclonal Anti-FLAG-Peroxidase is a purified immunoglobulin fraction of monoclonal Anti-FLAG (rat IgG1 isotype) isolated from culture supernatant of the 6F7 hybridoma cells grown in a bioreactor, conjugated to horseradish peroxidase (HRP). The hybridoma 6F7 was produced by the fusion of mouse myeloma cells and splenocytes from rat immunized with the FLAG peptide.

Monoclonal Anti-FLAG-Peroxidase, 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 product can be used for immunoblotting.

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 Cu^{2+} 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 Mg^{2+}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 0.01% merthiolate as a preservative.

Antibody concentration: 2–4 mg/mL
Molar ratio Ab/E: 0.6–1.5

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 dilution of 1:1000-1:2000 is recommended using extracts of transfected cells expressing C-terminal FLAG-tagged fusion protein.

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

Procedure for Immunoblotting

1. Separate FLAG-tagged fusion proteins from sample lysates using a standard SDS-PAGE protocol. Load 2.5–20 µg of total lysate protein per lane.
2. **Note**: The amount of extract to be loaded per slab or lane depends on the level of protein expression and may vary between experiments.

3. Transfer proteins from the gel to a nitrocellulose membrane.
4. Block the membrane using a solution of 5% non-fat dry milk in Dulbecco's phosphate buffered saline (PBS, Catalog Number D8537) at room temperature for 1 hour.
5. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (PBS-T, Catalog Number P3563) at room temperature.
6. Incubate the membrane with Monoclonal Anti-FLAG-Peroxidase using an optimized concentration in PBS containing 0.5% non-fat dry milk at room temperature with agitation for 2 hours.
7. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN®20 at room temperature.
8. Treat the membrane with a peroxidase substrate.

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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