

Product Information

Monoclonal Anti-FLAG®-Peroxidase produced in rat

Clone 6F7, purified immunoglobulin

SAB4200119

Product Description

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. The FLAG® peptide sequence, known also as DYKDDDDK, is one of the most widely used protein tags in recombinant protein expression and purification.¹ Protein tagging with the FLAG® tag may be done at the N-terminus, the N-terminus preceded by a methionine residue, the C-terminus, or at internal positions of the target protein. 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.

Monoclonal Anti-FLAG®-Peroxidase is a purified immunoglobulin fraction of monoclonal Anti-FLAG® (rat IgG1 isotype) isolated from culture supernatant of 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 which was immunized with the FLAG peptide.

Monoclonal Anti-FLAG®-Peroxidase, recognizes N-terminal, C-terminal and internal FLAG®-tagged fusion proteins. This product is especially recommended for identifying C-terminal FLAG®-tagged fusion proteins. The product can be used for immunoblotting. Several publications cite use of product SAB4200119 in their protocols.²⁻⁵

Reagent

This product is 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 (exact value on Certificate of Analysis for particular lot)

Molar ratio Ab/E

0.6-1.5 (exact value on Certificate of Analysis for particular lot)

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 that express C-terminal FLAG®-tagged fusion protein.

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

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Procedure

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.
Note: The amount of extract to be loaded per slab or lane depends on the level of protein expression and may vary between experiments.
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 (DPBS, 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-T, Cat. No. P3563) at room temperature.
5. 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.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 at room temperature.
7. Treat the membrane with a peroxidase substrate.

References

1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
2. Libby, E.A. *et al.*, *PLoS Genet.*, **11(6)**, e1005275 (2015).
3. Valsecchi, C.I.K. *et al.*, *Nat. Commun.*, **9(1)**, 3626 (2018).
4. Lee, D.H. *et al.*, *Nat. Commun.*, **11(1)**, 1838 (2020).
5. Valsecchi, C.I.K. *et al.*, *Nature*, **589(7840)**, 137-142 (2021).

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