Product Information

Monoclonal ANTI-FLAG® M5 antibody produced in mouse

Clone M5, purified immunoglobulin, buffered aqueous solution

F4042

Product Description

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. The *N*-terminal FLAG® peptide sequence contains a unique enterokinase cleavage site which allows it to be completely removed from the purified fusion proteins.

Monoclonal ANTI-FLAG® M5 is a purified IgG_1 monoclonal antibody that is isolated from mouse cell culture. This antibody binds to *N*-terminal Met-FLAG® fusion proteins. It is useful for detection of N-terminal Met-FLAG® fusion proteins expressed in mammalian and *Drosophilae* cells. M5 binding is not dependent on calcium. Several theses[2] and dissertations[3-8] cite of use of product F4042 in their protocols.

Note: this monoclonal ANTI-FLAG® M5 product is **not** recommended for detection of FLAG® fusion proteins that have been expressed in *E. coli*.

Product Profile

Protein concentration: 2.0-5.0 mg/mL (exact value on Certificate of Analysis for particular lot)

Antigenic binding site:
N-Met-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C

Specificity: Anti-FLAG® M5 monoclonal antibody detects an *N*-terminal Met-FLAG® fusion protein in a crude extract of mammalian cells with minimal cross reactivity.

Sensitivity: Anti-FLAG® M5 monoclonal antibody detects less than 1 ng of Met-FLAG-BAP™ (bacterial alkaline phosphatase) fusion protein on a dot blot using chemiluminescent detection.

Reagent

This product is supplied as a buffered aqueous solution with 10 mM sodium phosphate and 150 mM NaCl, pH 7.4, and also containing 0.02% (w/v) sodium azide.

Storage/Stability

Store undiluted antibody at –20 °C in working aliquots. Repeated freezing-and-thawing is **not** recommended.

Procedure for Western Blot

Note: To maximize detection sensitivity, adjust the Anti-FLAG® M5 antibody concentration as needed.

1. Transfer the *N*-terminal Met-FLAG•BAP™ or Met-FLAG® fusion protein of interest to a nitrocellulose membrane.
2. Block the membrane using a solution of 5% non-fat dry milk in TBS at 37 °C for 1 hour.
3. Wash the membrane twice for 1-2 minutes each in TBS at room temperature.
4. Incubate the membrane with Monoclonal ANTI-FLAG® M5 as the primary antibody at 10 µg/mL in TBS at room temperature for 30 minutes.
5. Wash the membrane three times for 1-2 minutes each in TBS at room temperature.
6. Incubate the membrane with Anti-Mouse IgG-Peroxidase as the secondary antibody at the manufacturer's recommended concentration in TBS. Incubate at room temperature for 30 minutes. Adjust the secondary antibody concentration to maximize detection sensitivity and to minimize background.

7. Wash the membrane three times for 15 minutes each in TBS at room temperature.
8. Treat the membrane with luminol sodium salt (5-Amino-2,3-dihydro-1,4-phthalazinedione sodium salt), Cat. No. A4685, or another peroxidase substrate.

References

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3. Zentner, Gabriel Etienne, "Genomic Analysis of Ribosomal DNA and Its Application to the Investigation of Disease Pathogenesis". Case Western Reserve University, Ph.D. dissertation, pp. 139-142 (January 2012).
4. Liburd, Janine Desirée, "Structural Insights into *Dictyostelium discoideum* Myosin Light Chain Specificity". Queen's University, Ph.D. dissertation, p. 63 (January 2013).
5. Sivadasan, Rajeeve, "The role of RNA binding proteins in motoneuron diseases" ("Die Rolle von RNA-bindenden Proteinen in Motoneuronerkrankungen"). Julius-Maximilians-Universität Würzburg and University Hospital, Würzburg, Ph.D. dissertation, p. 37 (2015).
6. Todd, Matthew Andrew Melville, "Characterizing the Cellular Role of PHF6". University of Ottawa, Ph.D. dissertation, p. 88 (2015).
7. Head, PamelaSara Elbaz, "Sirtuin 2 is a Human Tumor Suppressor and a Novel Regulator of the Non-Homologous End Joining Repair Pathway". Emory University, Ph.D. dissertation, pp. 42, 81 (2018).
8. Holodkov, Nikola, "Glutamic acid metabolism alterations, causes and consequences in ALS". The Open University, Ph.D. dissertation, p. 162 (September 2018).

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