Product Information

Monoclonal ANTI-FLAG® M1 Antibody produced in mouse

Clone M1, purified immunoglobulin, buffered aqueous solution

F3040

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® M1 antibody is a purified immunoglobulin (IgG_2b) that is isolated from mouse ascites fluid. This antibody binds to proteins with a FLAG® tag at the free *N*-terminus. It is useful to identify FLAG®-tagged proteins at the free *N*-terminus by common immunological applications in *E. coli*, yeast, and animal cells. The antibody is also useful for calcium-mediated affinity purification of FLAG®-tagged proteins at the free *N*-terminus when bound to a solid support. M1 antibody/antigen binding is dependent on calcium. Several theses[2] and dissertations[3-13] cite of use of product F3040 in their protocols.

Product Profile

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

Specificity: The antibody detects a single band of protein on a Western blot from an *E. coli* crude cell lysate or two bands from a *BJ3505* yeast crude extract which expresses a FLAG-BAP™ (bacterial alkaline phosphatase) fusion protein with minimal cross reactivity. The two bands from the yeast extract represent two different glycosylated forms of FLAG-BAP™.

Sensitivity: The antibody detects 1 ng of FLAG-BAP™ fusion protein on a dot blot, using chemiluminescent detection.

Reagent

This product is supplied in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide.

Storage/Stability

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

Procedure for Western Blot

1. Transfer the FLAG-BAP™ control protein or 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® M1 as the primary antibody at 10 µg/mL in TBS containing 1 mM $CaCl_2$ at room temperature for 30 minutes.
5. Wash the membrane three times, for 1-2 minutes each, in TBS containing 1 mM $CaCl_2$ at room temperature.
6. Incubate the membrane with Anti-Mouse IgG-Peroxidase as the secondary antibody at the manufacturer's recommended concentration in TBS containing 1 mM $CaCl_2$. Incubate at room temperature for 30 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
7. Wash the membrane three times for 15 minutes each in TBS containing 1 mM $CaCl_2$ 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.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

References

1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
2. Laakkonen, Johanna, "The Role of Proprotein Convertase Furin in the Activation of *N*-Glycosyltransferase Gnt-V." Tampere University, M.S. thesis, p. 30 (April 2020).
3. Wang, Hui, "The Roles of a LIM Domain Protein, HIC-5/ARA55, in TGF-b Signaling in Prostate Cancer Cells". Case Western Reserve University, Ph.D. dissertation, p. 95 (January 2009).
4. Iwamoto, Mari, "Utilizing Protein-Ligand Interactions To Control Biological Function". Stanford University, Ph.D. dissertation, p. 33 (June 2011).
5. Shola, Dorjee Tsewang Norbu, "The Roles of HIC-5 in BMP Signaling in Prostate Cancer Cells and Generation of Knockout Mouse Model". Case Western Reserve University, Ph.D. dissertation, p. 59 (August 2011).
6. Suo, Dong, "The role of Coronin-1 in neurotrophin signaling during sympathetic nervous system development". University of Virginia, Ph.D. dissertation, p. 189 (December 2014).
7. Majeed, Sophia Rafaa, "The mechanistic role of the clathrin light chain subunits in cell function". University of California San Francisco, Ph.D. dissertation, p. 83 (2015).
8. Christensen, Lea Cecile, "Insights on redox active proteins involved in ER-associated degradation". University of Copenhagen, Ph.D. dissertation, p. 28 (April 2016).
9. Patke, Aline, "Analysis of Signaling Mechanisms Essential to Mature B Cell Viability". Humboldt-Universität zu Berlin, Dr. rer. nat. dissertation, p. 36 (September 2017).
10. Caengprasath, Natarin, "Understanding the mechanisms regulating SCFA mediated release of anorectic gut hormones". Imperial College London, Ph.D. dissertation, p. 86 (January 2019).
11. Budzinska, Marta Izabela, "Investigating the mechanisms regulating retrograde transport and signalling of neurotrophin receptors in neuronal cells". University College London, Ph.D. dissertation, p. 75 (May 2019).
12. Alvarado, Guillermo Adrián Moya, "Mechanisms of long-distance control of dendritic growth by Brain-Derived Neurotrophic Factor (BDNF) in central neurons". Pontificia Universidad Católica de Chile, Ph.D. dissertation, p. 101 (October 2019).
13. Peng, Grace Eulan, "Elucidating the Role of Endocytosis in cAMP-dependent Transcription". University of California San Francisco, Ph.D. dissertation, p. 108 (2019).

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