

## Product Information

# Monoclonal ANTI-FLAG® BioM2 antibody produced in mouse

Clone M2, purified immunoglobulin, buffered aqueous glycerol solution

**F9291**

## 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.<sup>1</sup> 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® BioM2 is a purified mouse IgG<sub>1</sub> monoclonal antibody that is covalently attached to biotin by a hydrazide linkage. ANTI-FLAG® BioM2 will recognize the FLAG® sequence at the *N*-terminus, Met-*N*-terminus or *C*-terminus of FLAG® fusion proteins. The antibody can be detected by avidin or streptavidin conjugates. Monoclonal ANTI-FLAG® BioM2-Biotin is useful for Western blotting, microscopy applications, and formation of avidin-biotin complexes (ABC). Monoclonal ANTI-FLAG® BioM2-Biotin, in combination with an avidin or a streptavidin conjugate, is the preferred ANTI-FLAG® antibody for detection of FLAG® fusion proteins expressed in mammalian cells. Binding of the monoclonal antibody is not calcium-dependent.

Several theses<sup>2-5</sup> and dissertations<sup>6-20</sup> cite use of product F9291 in their protocols.

## Product Profile

Monoclonal ANTI-FLAG® BioM2-Biotin is formulated in 50% glycerol for added stability.

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

Protein concentration: ~1 mg/mL (exact value on Certificate of Analysis for particular lot)

**Dot blot:** the monoclonal antibody at the recommended concentration detects at least 2 ng of FLAG-BAP™ fusion protein, using chemiluminescent detection.

## Reagent

This product is supplied in buffered aqueous solution that contains 50% glycerol, 10 mM sodium phosphate (pH 7.25), and 150 mM NaCl, and also with 0.02% sodium azide present.

## Storage/Stability

Store undiluted antibody at -20 °C.

## Preparation Instructions

- Dilute the monoclonal antibody solution to 1-10 µg/mL in Tris Buffered Saline (TBS; 0.05 M Tris, pH 7.4, with 0.15 M NaCl).
- Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

## Procedure

### Procedure for Western Blot

1. Transfer the 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 30 minutes.
3. Wash the membrane twice for 5 minutes each in TBS at room temperature.
4. Incubate the membrane with Monoclonal ANTI-FLAG® BioM2-Biotin at 1-10 µg/mL in TBS for 30 minutes at room temperature.
5. Wash the membrane ten times for a total time of 10 minutes in TBS at room temperature. Incubate the membrane either with avidin-peroxidase conjugate (Cat. No. A3151), or with streptavidin-peroxidase conjugate (Cat. No. S5512) in TBS. For S5512, a concentration of 1 µg/mL is appropriate. Incubate at room temperature for 30 minutes. Adjust the conjugate concentration to maximize detection sensitivity and to minimize background.
6. Wash the membrane ten times for a total time of 10 minutes in TBS at room temperature.
7. Treat the membrane with a chemiluminescent peroxidase substrate.

### Procedure for immunostaining of cultured mammalian cells

1. Wash cells grown in a 9 cm<sup>2</sup> culture dish with 5 mL of TBS containing 1 mM calcium chloride (TBS/Ca).
2. Fix with 2 mL of a freshly prepared 1:1 mixture of acetone:methanol.
3. Wash four times with 2.5 mL of TBS/Ca.
4. Incubate with 10 µg/mL of Monoclonal ANTI-FLAG® BioM2-Biotin in TBS/Ca for 1 hour.
5. Wash five times with 2 mL of TBS/Ca.
6. Add avidin-peroxidase or streptavidin-peroxidase at a concentration of 1 µg/mL in TBS/Ca. Incubate 30 minutes at room temperature.
7. Wash five times with 2 mL of TBS/Ca.
8. Stain with a peroxidase substrate such as o-dianisidine dihydrochloride (Cat. No. D9154). Monitor the staining by microscopy. Stop the reaction by washing with distilled water.

## References

1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
2. Zhao, Min, "Sequence Analysis of the *Leishmania Mexicana* Amastigote Specific Gene A600". University of British Columbia, M.S. thesis, p. 27 (December 2001).
3. Lajko, Michelle, "Examination of herpesvirus entry glycoprotein interactions using proximity biotinylation". DePaul University, M.S. thesis, p. 14 (August 2014).
4. Guo, Chenxi, "The role of JNK signalling in pancreatic cancer". University of Manchester, M. Phil. thesis, p. 33 (2016).
5. Moreno, Eva Domènech, "Identification of new protein-protein interactions with NUA2 by proximity-dependent biotin labeling". University of Helsinki, M.S. thesis, p. 16 (May 2017).
6. Yang, Chih-Chin (Eric), "Identification and characterization of proteins that interact with myocyte enhancer factor 2, E12, and smooth muscle LIM proteins". University of Toronto, Ph.D. dissertation, p. 92 (2000).
7. Lummerstorfer, Judith-Antonia, "The significance of the laminin/nidogen-1 interaction for basement membrane formation and stability in embryoid bodies". Universität zu Köln, Ph.D. dissertation, p. 64 (2001).
8. Happel, Christine M., "Molecular Basis for Mu-Opioid Regulation of Chemokine Gene Expression". Temple University, Ph.D. dissertation, p. 55 (May 2009).
9. Küch, Eva-Maria, "Intrazelluläre Kanalisierung von Fettsäuren durch Acyl-CoA-Synthetasen" ("Intracellular channeling of fatty acids through Acyl-CoA synthetases"). Ruprecht-Karls-Universität Heidelberg, Ph.D. dissertation, p. 26 (February 2012).
10. Hwang, Helen, "Structural Dynamics of Telomeric Overhang Accessibility". University of Illinois at Urbana-Champaign, Ph.D. dissertation, p. 39 (2013).
11. Ayhan, Fatma, "Repeat Associated Non-ATG (RAN) Translation in Spinocerebellar Ataxia Type 8". University of Florida, Ph.D. dissertation, p. 102 (2016).

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2021 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

F9291dat Rev 09/21 RS,PHC,GCY

For research use only. Not for use in diagnostic procedures.

12. Gyapon-Quast, Frederick, "Novel Glycomic approaches to unravel Protein-Carbohydrate Interactions in Complement-associated Kidney Disease". Imperial College London, Ph.D. dissertation, p. 72 (November 2016).
13. Ong, Edmund Wing, "Investigating the Effects of Prolonged Mu Opioid Receptor Activation upon Opioid Receptor Heteromerization". Queen's University (Kingston, Ontario, Canada), Ph.D. dissertation, pp. 132, 134 (March 2017).
14. Seegel, Julia, "Assessing possible protective functions and interaction partners of Progranulin". Heinrich-Heine-Universität Düsseldorf, Ph.D. dissertation, p. 35 (June 2017).
15. Myler, Logan Ross, "Single-Molecule Studies Reveal Mechanisms of Human DNA Double-Strand Break Repair". University of Texas at Austin, Ph.D. dissertation, p. 36 (May 2018).
16. Chen, Grace R., "Restoring and enhancing Argonaute2-catalyzed cleavage". Massachusetts Institute of Technology, Ph.D. dissertation, p. 73 (June 2018).
17. Knarston, Ingrid May, "Disorders of sex development: Genetic analysis and development of a novel *in vitro* cell model". University of Melbourne, Ph.D. dissertation, p. 33 (October 2018).
18. Pegg, Amy Katrina, "BMP signalling via Id proteins in mesoderm progenitor cell differentiation". University of Edinburgh, Ph.D. dissertation, p. 76 (November 2018).
19. Szyroka, Justyna, "Regulation of the 'molecular scissor' ADAM10 by tetraspanin Tspan15". University of Birmingham (England, UK), Ph.D. dissertation, p. 52 (May 2019).
20. Shoaib, Ekram Abdullah, "Biochemical and genetic characterisation of ciliary transition zone transmembrane proteins in cystic kidney disease and ciliopathies". University of Leeds, Ph.D. dissertation, pp. 124, 125 (May 2020).

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

## Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://www.sigmaaldrich.com/techservice).

## Standard Warranty

The applicable warranty for the products listed in this publication may be found at [SigmaAldrich.com/terms](https://www.sigmaaldrich.com/terms).

## Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://www.sigmaaldrich.com/offices).

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2021 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

F9291dat Rev 09/21 RS,PHC,GCY

For research use only. Not for use in diagnostic procedures.