Technical Bulletin

ANTI-FLAG® M2-Peroxidase (HRP) Antibody, Mouse Monoclonal

Clone M2, purified immunoglobulin, buffered aqueous glycerol solution

A8592

Product Description

Monoclonal ANTI-FLAG® M2-Peroxidase is a covalent conjugate of a purified IgG1 monoclonal ANTI-FLAG® M2 antibody, which has been isolated from a mouse cell culture,[1] with horseradish peroxidase (HRP). This antibody-HRP conjugate binds to FLAG® fusion proteins and recognizes the FLAG® epitope at any position in the fusion protein (N-terminal, Met-N-terminal, C-terminal, or internal FLAG® peptides). This conjugate is useful for identification of FLAG® fusion proteins by common immunological procedures.

Monoclonal ANTI-FLAG® M2-Peroxidase may be used for the detection of FLAG® fusion proteins. Applications for the conjugate include Western blots, dot blots, ELISA, and immunocytochemistry. Several theses[2,3] and dissertations[4-55] cite use of this product in their protocols.

Reagent

This product is supplied as a solution in 10 mM sodium phosphate, pH 7.4, containing 150 mM NaCl and 50% glycerol, plus stabilizer and preservative.

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

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.

Preparation Instructions

Dilute ANTI-FLAG® M2-Peroxidase solution to the recommended working dilution in Tris-Buffered Saline (TBS; 0.05 M Tris, pH 7.4, with 0.15 M NaCl).

Product Profile

- Suggested working dilutions:
  - Immunocytochemistry and Western blotting: an antibody titer of 1:100 to 1:1000 may be used.
  - ELISA: the suggested minimum antibody titer is 1:20,000.
- Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
- To obtain optimal results, it is recommended that each individual user determine the working dilution by titration assay.

Procedure

Procedure for Western Blot

1. Carry out SDS-PAGE of FLAG® fusion protein and transfer the protein to a PVDF membrane (such as Immobilon®-P).
2. Block the membrane with 5% nonfat milk (M7409) in TBS with 0.05% TWEEN® 20 detergent (P9416) at room temperature for 1 hour.
3. Wash the membrane in TBS with 0.05% TWEEN® 20 detergent twice for 5 minutes each.
4. Incubate the membrane with ANTI-FLAG® M2-HRP titered at 1:100 to 1:1000 in TBS with 0.05% TWEEN® detergent 20 at room temperature for 1 hour.
5. Wash the membrane in TBS with 0.05% TWEEN® 20 detergent six times for 5 minutes each.
6. Treat the membrane with luminol, sodium salt (A4685) or other peroxidase substrate to detect the FLAG® fusion protein.

Procedure for ELISA

This procedure is based on direct adsorption of the target protein onto an ELISA plate. In some cases, target proteins may not adsorb efficiently, and a primary antibody directed against the target protein may first be adsorbed to provide for subsequent immobilization of target protein.

1. Prepare the FLAG® fusion protein sample at 1-10 µg/mL in 0.1 M $NaHCO_3$, pH 9.5. Use higher concentrations for crude preparations and lower concentrations for purified proteins.
2. Coat plate overnight with 100-200 µL of the FLAG® fusion protein sample at 2-8 °C.
3. Rinse plate with TBS with 0.05% TWEEN® 20 detergent (TBS-T) three times.
4. Block plate with 100-200 µL of 1% non-fat dried milk (M7409) at room temperature for one hour.
5. Incubate the plate with 100-200 µL of ANTI-FLAG® M2-HRP titered at a minimum of 1:20,000 at room temperature for one hour.

   Note: To obtain optimal results, it is recommended that each individual user determine the suitable working dilution by titration assay.
6. Rinse plate with TBS-T five times.
7. Incubate the plate with 100-200 µL of SIGMA*FAST*™ OPD (*o*-phenylenediamine dihydrochloride) substrate (P9187 or equivalent) at room temperature for 30 minutes.
8. Stop reaction with 50 µL of 1 N HCl.
9. Read plate at 450 nm.

Storage/Stability

The product should be stored at −20 °C. Once diluted, repeated freezing and thawing is **not** recommended.

References

1. Brizzard, B.L. *et al.*, *BioTechniques*, **16(4)**, 730-735 (1994).
2. Chun, Sophia Weng Shum, "Overexpression of the transcription factor ZFP60 in a multipotential mesenchymal cell line and its effects on gene expression and cell differentiation potential". University of Toronto, M.Sc. thesis, p. 38 (2008).
3. Duncan, Aundrietta DeVan, "Dissecting the Interaction between p53 and TRIM24". University of Texas Health Science Center at Houston/M.D. Anderson Cancer Center, M.S. thesis, p. 16 (2011).
4. Koues, Olivia I., "The Epigenetic Regulation of Cytokine Inducible Mammalian Transcription by the 26S Proteasome". Georgia State University, Ph.D. dissertation, p. 79 (2009).
5. Dorn, Karolin Viktoria, "Evc2 regulates Hedgehog Signaling at Primary Cilia". Heinrich-Heine-Universität Düsseldorf, Ph.D. dissertation, p. 27 (September 2012).
6. Liu, Xiaoyan, "Identification of sumoylated proteins and investigation of protein ubiquitination in the NF-κβ pathway". University of Kentucky, Ph.D. dissertation, pp. 39, 79, 82, 121, 122 (2012).
7. Fabian, Anne-Katrin, "InterAKTion with FKBPs: Modulation of the Akt/mTOR pathway by FKBPs". Ludwig-Maximilians-Universität München, Ph.D. dissertation, p. 35 (2013).
8. Jäger, Julia Maria, "The Role of Hepatic Transforming Growth Factor Beta1-stimulated Clone 22 D1 (TSC22D1) in the Regulation of Systemic Cholesterol Metabolism". Ruperto-Carola University (Heidelberg, Germany), Dr. rer. nat. dissertation, p. 90 (2013).
9. Hsiau, Timothy H., "Tools for engineering biology: methods for designing, building, and testing". University of California Berkeley, Ph.D. dissertation, p. 18 (2013).
10. Shaposhnikov, Dmitry, "The role of myocardin-related transcription factors in proliferation and cell cycle regulation of fibroblast cells". Martin-Luther-Universität Halle-Wittenberg, Dr. rer. nat. dissertation, p. 26 (April 2013).
11. Ho, Jessica Sook Yuin, "Chromatin Control of the Antiviral Response to Influenza". Rockefeller University, Ph.D. dissertation, p. 34 (June 2014).
12. Renberg, Rebecca Lynn, "Characterization of heme acquisition in *Leishmania*". University of Maryland College Park, Ph.D. dissertation, p. 67 (2014).
13. Bhatia, Sanil, "A role of nucleolin in human hematopoietic progenitor cells". Heinrich-Heine-Universität Düsseldorf, Ph.D. dissertation, p. 36 (December 2014).

14. Craig, Alexander William, "Molecular Genetic Analysis of FAM58A and Expansion of the Mutation Spectrum in STAR Syndrome". Albert-Ludwigs-Universität (Freiburg im Breisgau, Germany), Ph.D. dissertation, p. 27 (February 2015).
15. Fuhrmeister, Jessica, "The role of hepatic Growth Arrest and DNA Damage-inducible 45 beta (GADD45β) in adaptive metabolic control". Ruperto-Carola University (Heidelberg, Germany), Dr. rer. nat. dissertation, p. 147 (March 2015).
16. Naqvi, Ammar S., "Temporal patterns of short non-coding RNA modifications and expression". Rutgers, The State University of New Jersey, Ph.D. dissertation, p. 138 (May 2015).
17. Pavel-Danu, Mara, "Biochemical analysis of engineered TAL Effector Nuclease at ON and novel OFF target sites". Stanford University, Ph.D. dissertation, p. 67 (May 2015).
18. Chu, Yanjie, "Surface Remodeling of Lentiviral and Adeno-Associated Viral Vectors via Metabolically Introduced Bioorthogonal Functionalities". Stony Brook University, Ph.D. dissertation, pp. 19, 20, 29, 32, 57, 73 (August 2015).
19. Manning, Benjamin Jon, "ATP-Dependent eterochromatin Remodeling". University of Massachusetts Medical School, Ph.D. dissertation, pp. 94, 95 (September 2015).
20. Kennedy, Justine Elizabeth, "Regulation of CXCR4 Intracellular Trafficking by Ubiquitin". Loyola University Chicago, Ph.D. dissertation, p. 92 (December 2015).
21. Godin, Stephen Kenneth, "The Shu complex is a conserved regulator of Rad51 filament formation". University of Pittsburgh, Ph.D. dissertation, p. 95 (2015).
22. Harms, Matthew James, "Controlling Thermogenesis: Understanding the Role of PRDM16 in the Development and Function of Brown Fat". University of Pennsylvania, Ph.D. dissertation, p. 83 (2015).
23. Poshusta, Garrett Robert, "Pathogen host interactions: proteomics of Influenza NEP during infection reveals an antagonistic role in the formation of tight junctions". University of Washington, Ph.D. dissertation, p. 37 (2015).
24. Rubel, Carrie E., "Defining the Molecular Mechanisms of Ubiquitin Proteasome System Dysfunction as a Driver of Disease: CHIP mutation in SCAR16". University of North Carolina Chapel Hill, Ph.D. dissertation, pp. 94, 182 (2015).
25. Tseng, Hui-yuan, "Identification and characterization of conformation-specific cytoplasmic integrin interactors". Ludwig-Maximilians-Universität München, Ph.D. dissertation, p. 112 (2015).
26. Vaughn, Lauren S., "Regulation of Stress Response and InnateImmunity by DSRNRNAA-Binding Proteins PACT andTRBP". University of South Carolina – Columbia, Ph.D. dissertation, pp. 25, 49 (2015).
27. Ayhan, Fatma, "Repeat Associated Non-ATG (RAN) Translation in Spinocerebellar Ataxia Type 8". University of Florida, Ph.D. dissertation, pp. 98, 102 (2016).
28. Srivastava, Shashank, "Regulation of Alteration/Deficiency in Activation 3 (ADA3) by Acetylation and its Role in Cell Cycle Regulation and Oncogenesis Acetylation and its Role in Cell Cycle Regulation and Oncogenesis". University of Nebraska Medical Center, Ph.D. dissertation, pp. 29, 66, 123 (December 2016).
29. Kanai, Stanley Michinobu, "Mechanisms of G Protein Regulation by RGS Proteins and Small Molecule Inhibitors". Washington University in St. Louis, Ph.D. dissertation, p. 43 (May 2017).
30. Rachev, Ev, "Characterization of CFAP43 and its function in motile cilia". Medizinische Hochschule Hannover, Dr. rer. nat. dissertation, p. 92 (2017).
31. Roundtree, Ian A., "Post-transcriptional gene regulation by N^6-methyladenosine and selective RNA binding proteins". University of Chicago, Ph.D. dissertation, p. 54 (December 2017).
32. Purandare, Neeraja, "Chchd10, A Novel Bi-Organellar Regulator Of Cellular Metabolism: Implications In Neurodegeneration Implications In Neurodegeneration". Wayne State University, Ph.D. dissertation, p. 80 (January 2018).
33. Lane, Elizabeth, "Regulation of Hepatic Fuel Utilization and Storage Through BAD-Dependent Glucose Signaling". Harvard University, Ph.D. dissertation, p. 66 (June 2018).

34. Tzakoniati, Foteini, "A Chemical Biology Approach to Understanding the Basis of Voltage-Gated Sodium Channel Modulation". Imperial College London, Ph.D. dissertation, p. 126 (2018).
35. Feng, Weiran, "BRCA2 suppresses replication stress through homologous recombination". Memorial Sloan-Kettering Cancer Center, Ph.D. dissertation, p. 50 (2018).
36. Lérias, Joana Ramos Rapaz, "Anoctamin 1 - A Member of a Novel Family of Ion Channels with Extended Functions and Significance in Disease". Universidade de Lisboa, Ph.D. dissertation, p. 26 (2018).
37. Nemani, Neeharika, "Molecular Determinant of Mitochondrial Shape Change". Temple University, Ph.D. dissertation, p. 26 (2018).
38. Smith, Eric Matthew, "Structural and Functional Insights into Telomerase Recruitment". University of Michigan, Ph.D. dissertation, p. 53 (2018).
39. Fu, Yanlin, "Proteostasis Maintenance of g-Aminobutyric Acid Type A Receptors ($GABA_ARs$)". Case Western Reserve University, Ph.D. dissertation, p. 99 (May 2019).
40. Fulcher, Luke James, "Identification and characterisation of FAM83 proteins as key regulators of CK1 protein kinases in cells: FAM83D recruits CK1α to the mitotic spindle for proper spindle positioning". University of Dundee, Ph.D. dissertation, p. 68 (August 2019).
41. Gonzalez-Pecchi, Valentina, "Characterization of MYC Interaction with Nuclear Receptor SET Domain Protein 3 (NSD3)". Emory University, Ph.D. dissertation, pp. 38, 67 (2019).
42. Hähle, Andreas, "Elucidation of the molecular and cellular mechanisms of FKBP51-selective inhibitors" ("Aufklärung der molekularen und zellulären Mechanismen FKBP51-selektiver Inhibitoren"). Technischen Universität Darmstadt, Dr. rer. nat. dissertation, p. 21 (2019).
43. Hasselluhn, Marie Christin, "Nuclear NFATc1/Smad3 complexes in Smad4-deficient pancreatic cancer". Georg-August Universität Göttingen, Dr. rer. nat. dissertation, p. 30 (2019).
44. Htet, Zaw Min, "Mechanisms of Regulation of Cytoplasmic Dynein". Harvard University, Ph.D. dissertation, pp. 124, 134 (2019).
45. Madhusudhan, Nikhil, "Elucidating the target and selectivity of a non-small cell lung cancer toxin". University of Texas Southwestern Medical Center at Dallas, Ph.D. dissertation, p. 60 (2019).
46. Zieger, Hanna, "Investigation of disease-associated scaffold proteins in the postsynaptic density". Freie Universität Berlin, Dr. rer. nat. dissertation, p. 18 (2019).
47. Burnett, Samuel B., "Dysregulation of the Integrated Stress Response in Early Onset Dystonia (DYT16) Due to Mutations in Pact". University of South Carolina, Ph.D. dissertation, p. 67 (2020).
48. Cortez, Jessica T., "CRISPR screen in regulatory T cells reveals modulators of Foxp3". University of California San Francisco, Ph.D. dissertation, p. 44 (2020).
49. Gao, Minling, "EGFR activates a TAZ-driven oncogenic program in glioblastoma". Johns Hopkins University, Ph.D. dissertation, p. 70 (March 2020).
50. Hurd, Catherine, "Development of stapled peptides targeting the Ral GTPases". University of Cambridge, Ph.D. dissertation, p. 173 (September 2020).
51. Magee, Nancy Emole, "Uncovering Mechanisms Behind Simple Steatosis Progression to Non-Alcoholic Steatohepatitis". University of Kansas, Ph.D. dissertation, p. 26 (2020).
52. Ononye, Onyekachi Ebelechukwu, "Defining the role of lysine acetylation in regulating the fidelity of DNA synthesis". Purdue University, Ph.D. dissertation, p. 49 (December 2020).
53. Renner, Tyler, "Uncovering the Complexity of a Simple Retrovirus: A Study of Glycosylated Gag and Flow Virometry". University of Ottawa, Ph.D. dissertation, p. 94 (2020).
54. Storck, William K., "Characterization of LSD Complex Function, Histone Exchange, and Regulation of a Tryptophan Catabolism Gene Pair in *Neurospora crassa*". University of Oregon, Ph.D. dissertation, pp. 22, 37, 50 (2020).
55. Eckelmann, Bradley John, "XRCC1 plays diverse roles in DNA strand break repair and human disease". Texas A&M University, Ph.D. dissertation, p. 43 (May 2021).

General Western Blot References

56. Bjerrum, O.J., and Heegaard. N.H.H., *CRC Handbook of Immunoblotting of Proteins*, Volume I: Technical Descriptions. CRC Press, pp. 229-236 (1988).
57. Dunbar, B.S. (ed.), *Protein Blotting: A Practical Approach*. IRL Press at Oxford University Press (Oxford, UK/New York, NY), pp. 67-70 (1994).
58. Fortin, A. *et al*., *Biochem. Cell Biol*., **72(5-6)**, 239-243 (1994).
59. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY), pp. 726 (1989).
60. Pampori, N.A. *et al*., *BioTechniques*, **18(4)**, 589-590 (1995).

Western Blot Troubleshooting Guide[56-60]

Problem	Possible Cause	Solution
High Background	Too much conjugated antibody	Perform a titer of the conjugated antibody until an acceptable signal to noise ratio is obtained.
	Inappropriate blocking reagent	Increase the concentration of the blocking reagent by preparing the reagent with one-half the recommended volume of water. In addition, some antibodies may cross-react with certain blocking reagents. To test for this possibility, prepare a "blank" membrane that does not contain the primary antibody.
	Inappropriate blocking protocol	Increase the blocking time and/or increase the blocking temperature to 37 °C.
	Inappropriate wash protocol	Increase the number of washes. Consider using more stringent washes. For example, include 0.05% TWEEN® 20 detergent or 0.1% TRITON® X-100 detergent in the wash buffer.
	Overincubation in colorimetric substrate solution	Decrease the staining time. The membrane should be exposed to the colorimetric substrate until a positive signal is seen, but as the background begins to develop, the reaction should be stopped. For colorimetric substrates: • Incubate for 5-10 minutes, or whenever bands are visible. • The time required may be increased or decreased, but should not be longer than 60 minutes. For horseradish peroxidase substrates: wash the membrane with 0.1% sodium azide with 1% SDS in either TBS (Tris-Buffered Saline) or PBS (Phosphate Buffered Saline) to stop the reaction.
	Inappropriate film	Switch to film designated for chemiluminescent detection such as BioMax® Light, MS, and MR.
Extraneous spots	Aggregated protein or antibody conjugate	Centrifuge the conjugate solution at 10,000 × *g* for 10 minutes and use the supernatant.

Problem	Possible Cause	Solution
No signal	FLAG® not expressed on fusion protein.	Verify the nucleic acid sequence of FLAG® in the vector construct.
	No target protein present on membrane.	Verify transfer by visualizing proteins on the membrane using a Ponceau S solution (P7170). If possible, a positive control should always be run to ensure components are functioning.
	Target protein poorly represented in sample.	Positive controls should always be included. If the positive control works, the sample may not contain the FLAG® fusion protein of interest, or it may be present at concentrations too low to detect. Immunoprecipitation with ANTI-FLAG® M2 Affinity Gel (A2220) may be required for low FLAG® fusion protein concentrations. Positive controls available at SigmaAldrich.com: Amino-terminal FLAG-BAP™ Fusion Protein (P7582) Carboxy-terminal FLAG-BAP™ Fusion Protein (P7457) Amino-terminal Met-FLAG-BAP™ Fusion Protein (P5975)
	Overblocking such that antigen is covered by blocking reagent.	Masking of a signal can occur if the blocking reagent, such as the casein or gelatin blocking buffers (C7594 or G7663, respectively) is used at too high a concentration. A dilution of 1:1 to 1:3 may be done to decrease the concentration. If the problem persists, different blocking reagents should be tried.
	Inadequate exposure time using chemiluminescence system.	First exposure should be 1 minute. If no signal is seen, expose for longer times. It is recommended to try 5 minutes, 10 minutes, etc. If excess signal is seen, try as short an exposure as practical (down to 1 second) without using a cassette.
	Antibody concentration is not optimal.	Determine optimal working dilution for ANTI-FLAG® peroxidase conjugate by titration. Consider using more antibody if no signal or weak signal is detected. Also, antibody used at too high a concentration can also cause inhibition of signal, especially in chemiluminescent detection systems.
	Substrate solution is inappropriate for horseradish peroxidase.	Choose substrate recommended for use with horseradish peroxidase such as luminol for chemiluminescent detection or SIGMA*FAST*™ 3,3'-Diaminobenzidine (DAB, D4418), 3-Amino-9-Ethylcarbazole (AEC, A6926), or 4-Chloro-1-Naphthol (4C1N: C6788, tablet; or C8302, solution) for brown, red, or blue colorimetric end products, respectively.
	Enzyme conjugate may have lost enzymatic activity if old or improperly stored.	Determine if the enzyme conjugate is active.

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