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Product Information

Anti-FLAG®-Peroxidase antibody produced in rabbit
IgG fraction of antiserum

Product Number **H7425**

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. Tagging with the FLAG® peptide sequence may be done at the N-terminus, N-terminus preceded by a methionine residue, C-terminus, or at internal positions of the target protein. FLAG® may also be placed in association with other tags.[1] 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 allowing it to be completely removed from the purified fusion proteins. Cleavage catalyzed by Cu^{2+} ions of the C-terminal FLAG® peptide from a fusion protein has been reported.[2] A sequence motif with five out of eight amino acid residues identical to the FLAG® peptide is found in both rat and mouse Mg^{2+} dependent protein β-phosphatase,[3] as well as in the human and bovine enzyme.

Anti-FLAG® antibody is developed in rabbits using purified FLAG® fusion protein as immunogen. Whole antiserum is purified using protein A immobilized on agarose to provide the IgG fraction of the antiserum and is conjugated to horseradish peroxidase.

ANTI-FLAG® recognizes the FLAG® epitope located on FLAG® fusion proteins.[4] The antibody reacts with N-terminal, N-terminal-Met, and C-terminal FLAG fusion proteins by immunoblotting. Specific staining is inhibited by the FLAG® peptide (N-Asp-Tyr-Lys-Asp-AspAsp-Asp-Lys-C). Applications for the conjugate include Western blots and ELISA.

Reagent

Supplied as a lyophilized powder.

Preparation Instructions

Reconstitute the content of the vial with 0.1 mL of distilled water to a final antibody concentration of ~4 mg/mL. After reconstitution, the solution contains 2.5% trehalose and 0.05% MIT in 0.01 M sodium phosphate buffered saline.

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.

Storage/Stability

Store the lyophilized product at 2–8 °C. For extended storage after reconstitution, keep at –20 °C in working aliquots. Avoid repeated freeze-thaw cycles. For continuous use after reconstitution, keep at 2–8 °C for up to 1 month. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a minimum working dilution of 1:2000 - 1:4000 detects amino-terminal FLAG®-BAP fusion protein in an *E. coli* crude cell lysate.

Note: In order to obtain best results in different techniques and preparations, we recommend determining optimal working concentration by titration test.

Procedure for Western Blot

1. Separate FLAG® fusion proteins from sample lysates using a standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk (Catalog Number M7409) in phosphate buffered saline (PBS, Catalog Number 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, Catalog Number P3563) at room temperature.
5. Incubate the membrane with ANTI-FLAG® Peroxidase-conjugated antibody titered at 1:2000 to 1:4000 in PBS with 0.05% TWEEN 20 at room temperature for 2 hours.
6. Wash the membrane in PBS with 0.05% TWEEN 20 three times for 5 minutes each.
7. Treat the membrane with a peroxidase substrate to detect the FLAG® fusion protein.

References

1. Robeva, A.S. *et al*., *Biochem. Pharmacol*., **51(4)**, 545- 555 (1996).
2. Humphreys, D.P. *et al*., *Protein Eng.*, **12(2)**, 179-184 (1999).
3. Schafer, K., and Braun, T., *Biochem. Biophys. Res. Commun*., **207(2)**, 708-714 (1995).
4. Chubet, R.G., and Brizzard, B.L., *Biotechniques*, **20(1)**, 136-141 (1996).

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Troubleshooting Guide.

Problem	Possible Cause	Solution
Fusion protein is not detected.	Possible Cause Solution Fusion protein is not detected. Protein is not expressed.	Verify FLAG® nucleic acid sequence in vector construct. If sequence is present, attempt to optimize expression.
	Target protein is 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 (Catalog Number A2220) may be required for low FLAG® fusion protein concentrations. Positive controls available: Amino-terminal FLAG®-BAP Fusion Protein, Catalog Number P7582 Carboxy-terminal FLAG®-BAP Fusion Protein, Catalog Number P7457 Amino-terminal Met-FLAG®-BAP Fusion Protein, Catalog Number P5975
	Detection reagents are defective.	Run appropriate controls to ensure performance. Use 0.5 μg/lane of a control FLAG®-BAP-fusion protein as a positive control. If no signal is obtained with the control, repeat the procedure using a newer lot of antibody-HRP conjugate and freshly prepared reagents.
	Inadequate exposure time using chemiluminescent system.	If no signal is seen, expose for longer times. 30-second to 10-minute exposure times are recommended.
	No target protein present on membrane.	Verify transfer by visualizing proteins on the membrane using a Ponceau S solution (Catalog Number P7170). Whenever possible, include a positive control to ensure components are functioning. Pre-stained protein markers (e.g. Catalog Number C1992) may also be used to verify complete transfer.
	Antigen is covered by blocking reagent due to over blocking.	Masking of a signal can occur if the blocking reagent (such as casein or gelatin blocking buffer, Catalog Numbers 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.
	Antibody concentration is not optimal.	Determine the optimal working dilution for ANTI-FLAG® antibody 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.
Cross-reactivity	Cellular extract concentration is too high.	2.5–10 μg per lane of total lysate protein is usually enough to obtain a good signal. Load less cellular extract, or serially dilute the cell extract to obtain the optimal signal-to-noise ratio.
	Antibody concentration is too high.	Use higher dilutions of the antibody.
	Antibody cross-reacts with naturally occurring epitopes similar to the FLAG® sequence.	Increasing the temperature to 37 °C during the blocking, binding, and wash steps may reduce cross-reactivity. Lysates from mock-transfected controls (transfected with plasmid without insert DNA) will help distinguish the FLAG® fusion proteins from other cross-reacting proteins.