SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

ANTI-FLAG®
produced in rabbit, affinity isolated antibody

Catalog Number **F7425**
Storage Temperature –20 °C

Product Description

ANTI-FLAG® is a rabbit IgG antibody that has been affinity-purified using the immunizing peptide immobilized on agarose. Polyclonal antibodies to the FLAG® sequence are useful tools for localization and characterization of FLAG fusion proteins.

ANTI-FLAG recognizes the FLAG epitope located on FLAG fusion proteins.[1] The antibody reacts with N-terminal,
N-terminal-Met, and C-terminal FLAG fusion proteins by dot blot analysis and immunoblotting. Specific staining is inhibited by the FLAG peptide (N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C). The ANTI-FLAG antibody immunoprecipitates FLAG fusion proteins from crude cell lysates. The antibody also reacts with transiently transfected cells expressing a FLAG fusion protein by indirect immunofluorescent staining.

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.[2] 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.[3]

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,[4] as well as in the human and bovine enzyme.

Reagent

Supplied as a solution in 10 mM phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative.

The antibody concentration is ~0.8 mg/ml.
See Certificate of Analysis for lot-specific value.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2–8 °C for up to one month. For extended storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A minimum antibody concentration of 2.5 μg/ml detects ≤2 ng of amino-terminal-FLAG-BAP™, amino-terminal Met-FLAG-BAP, and carboxy-terminal FLAG-BAP (bacterial alkaline phosphatase) fusion proteins by dot blot assay using a chemiluminescent substrate.

In immunoblotting, a minimum antibody concentration of 2.5 μg/ml detects amino-terminal FLAG-BAP fusion protein in an *E. coli* crude cell lysate and 1 ng of N-terminal FLAG-BAP fusion protein spiked in COS-7 whole cell extract.

A minimum antibody concentration of 5 μg/ml detects FLAG fusion protein in methanol-acetone fixed transiently transfected cells by indirect immuno-fluorescent.

At least 8 μg of the antibody immunoprecipitates amino-terminal FLAG-BAP fusion protein from 0.3–0.6 mg of *E. coli* crude lysate.

Procedure

Note: In order to obtain the best results and assay sensitivity with various techniques and preparations, determining optimal working dilutions by titration is recommended.

Procedure for Immunoblotting

1. Separate FLAG fusion proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5–20 μg of total lysate protein per lane.

2. Transfer proteins from the gel to a nitrocellulose membrane.

3. Block the membrane using a solution of 5% non-fat dry milk 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 antibody as the primary antibody using an optimized concentration in PBS containing 1% bovine serum albumin (BSA, Catalog Number A9647) at room temperature with agitation for 2 hours.
   Note: Using less ANTI-FLAG antibody may help to reduce background and cross-reactivity.

6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20 at room temperature.

7. Incubate the membrane with Anti-Rabbit IgG (whole molecule)-Peroxidase (Catalog Number A0545) as the secondary antibody at the recommended concentration in PBS containing 0.05% TWEEN 20. Incubate at room temperature for 1 hour. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20 at room temperature.

9. Treat the membrane with a peroxidase substrate.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

1. Grow transfected cultured cells expressing the FLAG fusion protein of choice on sterile coverslips on slides at 37 °C.

2. Wash the cells briefly in PBS.

3. Fix the cells in cooled methanol for 10 minutes at –20 °C and then in cooled acetone for 1 minute at –20 °C.

4. Wash the fixed cells twice in PBS (5 minutes each wash).

   Note: Blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibodies.

5. Incubate the fixed cells cell-side-up with ANTI-FLAG antibody as primary antibody using an optimized concentration in PBS. Incubate at room temperature for 1 hour.

6. Wash the fixed cells three times in PBS (5 minutes each wash).

7. Incubate the fixed cells cell-side-up with Anti-Rabbit IgG (whole molecule)-FITC (Catalog Number F9887) as the secondary antibody at the recommended concentration in PBS containing 1% BSA. Incubate at room temperature for 30 minutes.

8. Wash three times in PBS (5 minutes each wash).

9. Cover the cells using a coverslip with aqueous mounting medium and examine using a fluorescence microscope with appropriate filters.

Troubleshooting Guide

Problem	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 from Sigma: 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 10 ng/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 chemi-luminescent system	If no signal is seen, expose for longer times. 30 second to 10 minute exposure times are recommended.
	Inappropriate film used.	Switch to film designated for chemiluminescent detection such as BioMax™ Light.
	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. Prestained protein markers (Catalog Numbers C1992 and C4861) may also be used to verify complete transfer.
	Antigen is covered by blocking reagent due to overblocking.	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 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 concen-tration is too high.	Use higher dilutions of the antibody.
	Secondary antibody concentration is too high.	Use higher dilutions of the secondary 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.

References

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

FLAG and ANTI-FLAG are registered trademarks and FLAG-BAP is a trademark of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.
TWEEN is a registered trademark of Croda International PLC.
BioMax is a trademark of the Eastman Kodak Company

Ah,PHC 09/10-1

Sigma brand products are sold through Sigma-Aldrich, Inc.
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.
Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.
Please see reverse side of the invoice or packing slip.