

Data Sheet

ANTI-FLAG® Antibody produced in rabbit

Affinity isolated antibody, buffered aqueous solution

F7425

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. The FLAG® peptide sequence, N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C (known also as DYKDDDDK), is one of the most widely used protein tags in recombinant protein expression and purification.¹

Tagging with the FLAG® peptide sequence 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. 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 that allows it to be completely removed from the purified fusion proteins. Cleavage catalyzed by Cu²⁺ ions of the C-terminal FLAG® peptide from a fusion protein has been reported.³

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.⁴ 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. 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. Several theses⁵⁻⁶ and dissertations⁷⁻²⁶ cite use of this product in their research protocols.

Reagent

This product is 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. See the Certificate of Analysis for the lot-specific value of the antibody concentration.

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.

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.

Product Profile

Dot blot

A minimum working concentration of 1-2.5 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 using a chemiluminescent substrate.

Immunoblotting

A minimum working concentration of 1-2.5 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.

Indirect Immunofluorescence

A minimum working concentration of 5-10 µg/mL detects FLAG® fusion protein in methanol-acetone fixed transiently transfected cells.

Immunoprecipitation

A minimum working amount of 4-8 µg of the antibody immunoprecipitates 0.25-0.5 µg of FLAG®-tagged fusion protein.

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, Cat. No. 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, Cat. No. 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, such as Cat. No. 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-T at room temperature.
7. Incubate the membrane with Anti-Rabbit IgG (whole molecule)-Peroxidase (Cat. No. A0545) as the secondary antibody at the recommended concentration in PBS-T. 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-T 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 first 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 (Cat. No. 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. Examine using a fluorescence microscope with appropriate filters.

References

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Troubleshooting Guide

Problem	Possible Cause	Solution
	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.	<p>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.</p> <p>Immunoprecipitation with ANTI-FLAG® M2 Affinity Gel (Cat. No. A2220) may be required for low FLAG® fusion protein concentrations.</p> <p>Positive controls available from Sigma:</p> <ul style="list-style-type: none"> • Amino-terminal FLAG-BAP™ Fusion Protein (Cat. No. P7582) • Carboxy-terminal FLAG-BAP™ Fusion Protein (Cat. No. P7457) • Amino-terminal Met-FLAG-BAP™ Fusion Protein (Cat. No. P5975)
	Detection reagents are defective.	<p>Run appropriate controls to ensure performance.</p> <ul style="list-style-type: none"> • 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.
Fusion protein is not detected.	Inadequate exposure time using chemiluminescent 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.	<ul style="list-style-type: none"> • Verify transfer by visualizing proteins on the membrane using a Ponceau S solution (Cat. No. P7170). • Whenever possible, include a positive control to ensure components are functioning. • Prestained protein markers (such as Cat. No. C1992) 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 blocking buffer or gelatin blocking buffer, Cat. Nos. 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.	<ul style="list-style-type: none"> • 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.
	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.
Cross-reactivity	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.	<ul style="list-style-type: none"> • 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.

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