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 Cu^{2+} 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 theses[5-6] and dissertations[7-26] 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
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 (Cat. No. A2220) may be required for low FLAG® fusion protein concentrations. Positive controls available from Sigma: • 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.	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 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.	• 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.	• 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.
Cross-reactivity	Antibody concentration 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.

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