Product Information

# ANTI-FLAG® antibody, Rat monoclonal

Clone 6F7, purified from hybridoma cell culture

**SAB4200071**

## 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, known also as DYKDDDDK, is one of the most widely used protein tags in recombinant protein expression and purification.[1] Protein tagging with the FLAG® tag 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. 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.

ANTI-FLAG® antibody, Rat monoclonal (rat IgG1 isotype) is derived from the hybridoma 6F7, which is produced by the fusion of mouse myeloma cells and splenocytes from rat immunized with the FLAG® peptide. This antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

This ANTI-FLAG® antibody recognizes N-terminal, C-terminal and internal FLAG®-tagged fusion proteins. This product is especially recommended for identifying C-terminal FLAG®-tagged fusion proteins. The antibody may be used in various immunochemical techniques, including immunoblotting and immunoprecipitation. Several dissertations[2-4] cite of use of this product in their protocols.

## Reagent

This product is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: ~ 1.0 mg/mL (exact value on Certificate of Analysis for particular lot)

## Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze at -20 °C in working aliquots. 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

**Immunoblotting**: a working antibody concentration of 0.5-1.0 µg/mL is recommended using extracts of transiently transfected cells that express C-terminal-FLAG®-tagged protein.

**Immunoprecipitation**: a working antibody amount of 2.5-5.0 µg is recommended using lysates of transiently transfected cells that express C-terminal-FLAG®-tagged protein.

Note: To obtain the best results and assay sensitivity with various techniques and preparations, we recommend determining optimal working dilutions by titration.

## Procedure

### Procedure for Immunoblotting

1. Separate FLAG®-tagged 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 Dulbecco's Phosphate Buffered Saline (DPBS, 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-TWEEN® 20, 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 0.5% NFDM at room temperature with agitation for 2 hours.
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-Rat IgG- Peroxidase (Cat. No. A9542) 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.

**Note**: Using less Anti-FLAG® antibody may help to reduce background and cross-reactivity.

### Procedure for Immunoprecipitation

**Note**: The amount of cell lysate to be used for immunoprecipitation depends on the expression level of the tagged protein and the specific application.

1. To 0.1 to 1.0 mL of a cell lysate containing FLAG®-tagged protein, add Anti-FLAG® antibody. Incubate on a rotator for 2 hours to overnight at 4 °C (see **Note** above).
2. Centrifuge 20-40 µL Protein G-agarose beads (Cat. No. P3296) for 1 min 12,000 x *g*. Then wash twice with 1 mL RIPA buffer (50 mM Tris Base, 0.25 % w/v Deoxycholate, 1% IGEPAL®, 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4 °C.
3. Add the mixture from Step 1 to the beads from Step 2. Incubate on a rotator for 2 hours at 4 °C.
4. Spin down the beads. Remove the supernatant.
5. Wash beads four times with 1 mL RIPA buffer and one time with PBS (Cat. No. D8537) by vortex and short spin.
6. Resuspend pellet in 30 µL 2X SDS-PAGE sample buffer. Boil sample for 5 minutes and spin down. The sample is ready to be loaded on a SDS-PAGE gel.

## References


1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
2. Brasch, Julia, "Structural and Biophysical Studies of Adhesive Binding by Classical Cadherins". Gottfried Wilhelm Leibniz Universität Hannover, Dr. rer. nat. dissertation, p. 49 (2011).
3. Thirouin, Zahra Simone, "BDNF and SUMO Pathway Modulate Gephyrin Scaffold to Downregulate GABAergic Transmission after Ischemia". University of Zürich, Dr. sc. nat. dissertation, p. 65 (2016).
4. Urakova, Nadezda, "Identifying Molecular Virulence Factors of Rabbit Haemorrhagic Disease Virus". University of Canberra, Ph.D. dissertation, p. 13 (January 2017).


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