Product Information

Monoclonal ANTI-FLAG® Antibody Produced in Rabbit

Clone SIG1-25, ascites fluid

F2555

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.

Protein 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.[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 which allows it to be completely removed from the purified fusion proteins.

Monoclonal ANTI-FLAG® (Rabbit IgG) is a rabbit monoclonal antibody derived from the hybridoma SIG1-25, which is produced by the fusion of rabbit myeloma cells and splenocytes from rabbits that have been immunized with the FLAG® peptide sequence conjugated to KLH. This monoclonal ANTI-FLAG® product reacts only with *N*-terminal FLAG® fusion proteins. This product may be used in ELISA, immunoblotting, and immunofluorescence staining. This antibody has been used to detect *N*-terminal FLAG® proteins by WB,[2-5] immunoprecipitation and immunofluorescence.[4]

Several dissertations cite of use of product F2555 in their protocols.[6-8]

Reagent

The product is provided as ascites fluid which contains 15 mM sodium azide as a preservative.

Product Profile

Immunoblotting: A working dilution of 1:250-1:500 is recommended, based on tests with extracts of transiently transfected cells that express *N*-terminal FLAG-tagged proteins.

Immunocytochemistry: A working dilution of 1:125-1:250 is recommended, based on tests with transiently transfected cells that express N-terminal FLAG-tagged proteins.

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

Storage/Stability

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

References

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2. Kua, H.-Y. *et al.*, *Nat. Cell Biol.*, **14(7)**, 727-737 (2012).

3. Qi, Q. *et al*., *J. Biol. Chem.,* **287(37)**, 31482–31493 (2012)
4. Chau, J.F.L. *et al*., *Nat. Commun.,* **3**, 836 doi:10.1038/ncomms1832 (2012).
5. Rieder, R. *et al*., *RNA Biol.,* **9(4)**, 520-531 (2012).
6. Rauch, Bernadette, "Functional analysis of multiple general transcription factors in *Sulfolobus acidocaldarius*". Universität Duisburg-Essen, Dr. rer. nat. dissertation, p. 29 (2013).
7. Whedon, Samuel D., "Chemical strategies for investigation of deubiquitinases". University of Washington, Ph.D. dissertation, p. 30 (2018).
8. Stacy, Andrew Jared, "TIP60 Regulation of ΔNp63α Promotes Cellular Proliferation". Wright State University, Ph.D. dissertation, p. 35 (2020).

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