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Product Information

Monoclonal Anti-Nedd8

Clone ND23

Purified Mouse Immunoglobulin

Product Number **N 2786**

Product Description

Monoclonal Anti-Nedd8 (mouse IgG2b isotype) is derived from the ND23 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 20-32 of human Nedd8 conjugated Keyhole Limpet Hemocyanin (KLH). The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Nedd8 recognizes human Nedd8 and does not cross react with human SUMO1 or human ubiquitin (Prod. No. U 5507). The epitope recognized by the antibody resides within amino acids 20-32 of human Nedd8. Applications include the detection of Nedd8 by ELISA, immunoblotting, and immunocytochemistry.

Ubiquitin-like proteins are an expanding family of proteins that includes UCRP, sentrin1/SUMO1, sentrin2, sentrin3, Rub1, and Nedd8. Nedd8 is a small protein (9 kDa) that shows the highest similarity to the ubiquitin protein (60% amino acid sequence identity). It was identified as one of multiple neural precursor cell-expressed developmentally down-regulated genes in mice.¹ Nedd8 mRNA is highly restricted to the heart and skeletal muscle in adult human tissues, and is down regulated in mouse embryos.^{1,2}

As in the ubiquitin pathway, Nedd8 is conjugated to its target proteins by specific E1 and E2 like enzymes. For example, Nedd8 is conjugated to Cul-4A, which belongs to the cullin family of proteins, by the APP-BP1/hUba3 heterodimer and hUbc12 (as the E1- and E2-like enzymes, respectively).³ Nedd8 was found to be conjugated only to proteins of the cullin family, among them Cul-1,-2,-3,-4A, -4B, and -5. Human Cul-1 belongs to the SCF (Skp1-Cullin-1/CDC53-F-box) complex that catalyzes the ubiquitination of IκBα, β-catenin, and p27. Conjugation of Nedd8 to Cul-1 activates the SCF complex. Therefore, Nedd8 conjugation has a physiological role in processes where the stability of NF-κB and p27 is important.^{4, 5}

Reagent

Monoclonal Anti-Nedd8 is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C for up to one month.

For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

A working dilution of 0.15-0.3 µg/ml is determined by immunoblotting of recombinant GST-Nedd8.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

1. Kumar, S., et al., Biochem. Biophys. Res. Commun., **195**, 393-399 (1993).
2. Kamitani, T., et al., J. Biol. Chem., **272**, 28557-28562 (1997).
3. Osaka, F., et al., Genes Dev., **12**, 2263-2268 (1998).
4. Laney, J. D., et al., Cell, **97**, 427-430 (1999).
5. Read, M. A., et al., Mol. Cell. Biol., **20**, 2326-2333 (2000).

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