MONOCLONAL ANTI-NTF2, CLONE 4F5
Purified Mouse Immunoglobulin

Product Number **N 9527**

ProductInformation

Product Description

Monoclonal Anti-NTF2 (mouse IgG2b isotype) is derived from the 4F5 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a A/J mouse immunized with a recombinant human NTF2.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-NTF2 specifically recognizes NTF2.[1] The epitope recognized by the antibody lies within the extreme C-terminus of NTF2.[1] The antibody may be used in ELISA,[1] immunoblotting (14 kDa),[1] immunoprecipitation,[1] immunocytochemistry (3.7% formaldehyde-0.2% Triton X-100, or 4% paraformaldehyde-methanol),[1] microinjection,[1] *in vitro* nuclear protein import assays[1] and blocking of NTF2 binding to Ran.[1] Reactivity has been observed with human,[1] monkey,[1] hamster,[1] rat,[1] mouse,[1] chicken,[1] and *Xenopus*.[1]

Entry of molecules to, and exit from the nucleus ("nucleocytoplasmic transport") are crucial processes for cell function. Both play an important role in the regulation of diverse cellular processes including growth factor-mediated signaling, stress responses, cell cycle control, gene transcription and translation.[2] Gene expression is a complex process that begins when transcription factors bind DNA and induce mRNA transcription. This process takes place in the nucleus where the DNA resides; however, for protein translation, the mRNA has to exit the nucleus to the cytoplasm and to reach the ribosomes.[3] Proteins that are translated in the cytoplasm and exert their effect in the nucleus (like transcription factors) have to cycle back and enter the nucleus.

Eukaryotic cells are equipped with machinery charged with the responsibility of transporting a vast number of molecules in and out of the nucleus in a rapid, accurate, and often regulated manner. The cargoes for this machinery are diverse, comprising proteins and more elaborate RNA–protein complexes (RNPs, ribonucloproteins). Proteins and RNAs are imported and exported through nuclear pore complexes (NPCs), supramolecular (125 MDa in vertebrates) channels that perforate the double bilayer of the nuclear envelope.[4] NPCs mediate the active transport of most proteins and RNAs, as well as the passive diffusion of ions and small proteins less than ~40 kDa.[5,6] Proteins to be targeted into the nucleus (often termed "cargo"), identify themselves to the nucleocytoplasmic transport machinery by signals called nuclear localization signals - NLSs, and nuclear export signals – NESs, that can be protein or RNA-based (mostly for NES), or a composite of the two. There are many different signals, and these signals in protein cargoes and in some RNP cargoes, are recognized by one or more members of the nuclear transport receptor family, which binds to them and transports them.[2]

Soluble transport factors mediate recognition of NLS-containing proteins and their translocation through the NPC in a multistep process.[2,5] In the cytoplasm, the import receptor heterodimer importin α/β forms an import complex with an NLS-containing protein and facilitates binding to the cytoplasmic surface of the NPC.[6,7] Subsequent passage of the import complex through the central gated channel of the NPC probably involves transient interactions between the import complex and multiple NPC proteins. Upon reaching the nuclear side of the NPC, binding of RanGTP to importin β triggers disassembly of the import complex and release of the NLS-containing protein into the nucleoplasm. Importin α and β are then recycled to the cytoplasm as RanGTP complex for subsequent import reactions.[8] Conversion of RanGTP to RanGDP in the cytoplasm releases importin α and β.

NTF2 (nuclear transport factor 2, also known as p10)[9,10] an evolutionarily conserved protein that is essential for growth in yeast,[11,12] plays an important role in nuclear protein import and Ran regulation. The properties of NTF2 suggest it may modulate the steady-state distribution of Ran.[13] Both are imported into the nucleus as stoichiometric components of the import complex, and are required for efficient import. NTF2 regulates Ran distribution in living cells, and NTF2-mediated Ran nuclear import is required for NLS-dependent protein import *in vitro*.[1] NTF2 binds directly to RanGDP but not RanGTP.[14] In addition to binding RanGDP, NTF2 also binds NPC proteins. Mutations in NTF2 that abolish

Ran binding do not affect binding to NPC proteins, which suggests that Ran and NPC proteins bind to different domains of NTF2. NTF2 binds directly to p62, as well as to other NPC proteins containing multiple FxFG peptide repeats.[10,14] These repeat-containing NPC proteins have been proposed to provide binding sites for import complexes during translocation through the NPC.

Monoclonal antibodies reacting specifically with NTF2 are useful tools in the study of NTF2 involvement in nuclear protein transport.

Reagent
Monoclonal Anti-NTF2 is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml

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 hazardous and safe handling practices.

Storage/Stability
For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. 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
A working concentration of 1 µg/ml to 2 µg/ml is determined by immunoblotting using a HeLa cell cytosol preparation.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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