ProductInformation

MONOCLONAL ANTI-LAP2 (TMPO)
CLONE 6E10
Purified Mouse Immunoglobulin

Product Number **L 3414**

Product Description
Monoclonal Anti-LAP2 (TMPO) (mouse IgG1 isotype) is derived from the 6E10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide (amino acids 29-50) common to the N-terminus of all TMPOs.[1] 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-LAP2 (TMPO) reacts specifically with human lamina associated polypeptide 2 (LAP2), also known as thymopoietin (TMPO). The epitope recognized by the antibody resides within the N-terminus (amino acids 29-50), common to all human and mouse LAP2/TMPOs (isoforms α, β, γ, δ, ε and ζ).[1] The product is useful in immunoblotting[1] and immunocytochemistry (3% paraformaldehyde-0.5% Triton X-100 fixation, staining of nucleus and nuclear membrane). The relative staining intensity of TMPO isoforms may vary in different preparations in immunoblotting [76 kDa (α), 50 kDa (singlet or a doublet, β), 45 kDa (ε), 42 kDa (δ), 38 kDa (singlet or a doublet, γ) and 25 kDa (ζ)].[1]

In eukaryotic cells, DNA replication, RNA processing, and ribosome assembly all occur in the nucleus, while protein synthesis occurs in the cytoplasm. These activities are physically separated by the nuclear envelope (NE). The NE is a large complex structure composed of outer and inner lipid bilayer membranes, nuclear pores, nuclear lamina, and chromatin. The nuclear lamina is located between the inner nuclear membrane and the peripheral chromatin. It is composed mainly of nuclear lamins and lamina-associated proteins (LAPs).[1,2] The inner nuclear membrane and the nuclear lamina are involved in organizing nuclear structure and regulating nuclear events including: nuclear organization, DNA replication, regulation of transcription,[3] nuclear assembly and disassembly, apoptosis, correct spacing of nuclear pores, as well as providing mechanical stability to the nuclear periphery and topological organization of chromatin.[4]

During apoptosis, lamins are major targets for the caspase family of proteases, which trigger nuclear lamina breakdown. Indeed, lamin degradation has been reported in different cell types in response to different apoptosis-inducing stimuli.[5] In order to perform these activities the inner nuclear membrane and the nuclear lamina contain a unique set of proteins, including lamin type A and B, otefin, YA, LAP1, emerin, lamin B receptor (LBR), and Lamina Associated Polypeptide 2/Thymopoietin (LAP2/TMPO).[6] The proteins of the inner membrane form a complex network of interactions between each other and with chromatin. LAP2/TMPO proteins are a family of proteins that are highly conserved in mammals and *Xenopus* and are putatively involved in functional nuclear architecture and cell cycle control. The expression of the various TMPO isoforms is ubiquitous, with higher levels in proliferative tissues.[6,7] In mammalian cells, six alternatively spliced TMPO isoforms, designated α, β, β', γ, δ, ε, and ζ have been isolated and characterized.[6,8] All of them share an identical N-terminal 186 amino acid domain.

TMPOα is the largest member of the TMPO family. It shares the N-terminal amino acids of all TMPOs but has a unique long C-terminus. This region lacks a transmembrane domain but contains a nuclear localization signal (NLS), a putative tyrosine phosphorylation site, and two perfect p34cdc2 kinase phosphorylation sites. This unique region suggests a different intracellular localization than that of the TMPOβ/LAP2-type isoforms. Thus, TMPOα is localized throughout the nuclear interior in interphase cells. During metaphase, it dissociates from chromosomes and becomes concentrated around the spindle poles, probably due to specific phosphorylation. The protein is relocated to chromosomes at early stages of nuclear reassembly during telophase.[1] It is therefore suggested that, similar to the TMPOβ/LAP2-type isoforms, TMPOα has a major role in the process of NE reassembly but in a different manner. TMPOα/LAP2 has a role in regulating the dynamics of the nuclear lamina and also in the interaction between TMPOβ/LAP2 and the nuclear lamina that is required for nuclear growth after mitosis.[9]

TMPOβ/LAP2 is a type II integral protein of the inner NE, which binds lamin B1 and chromosomes in a phosphorylation dependent manner. It can be divided roughly into four domains: a hydrophilic C-terminus domain, a hydrophobic transmembrane domain, a NE targeting and lamina-binding domain, and a chromatin-binding domain. As NE disassembly begins at prophase TMPOβ/LAP2 undergoes phosphorylation by mitotic factors, possibly by p34cdc2 kinase. This has been found to abolish its interphase binding to both lamin B1 and chromosomes. Consistently during late anaphase, TMPOβ/LAP2, as well as lamin-associated and non-associated vesicles, associates independently around chromatin to bind the surface of the decondensing chromosomes in order to complete NE reassembly. Thus, it is suggested that TMPOβ/LAP2 plays a key role in NE disassembly and reassembly during mitosis and linking chromatin to the NE in interphase. The lamin binding region of TMPOβ/LAP2 coincides with TMPOβ/LAP2 NE targeting domain and includes residues 298-373. This region also binds GCL (germ-cell-less), a BTB/POZ domain-containing protein.[3] TMPOβ/LAP2, either together with GCL or alone, can repress the transcriptional activity of the E2F-DP heterodimer.[3] The chromosome binding site of TMPOβ/LAP2 resides within residues 1-85, a region which is common to all TMPO isoforms. Emerin, a ubiquitous integral nuclear membrane protein (IMP) of the inner nuclear membrane, has a sequence and structural homology to TMPOβ/LAP2.[10]

Three other isoforms of the TMPO family, TMPOs γ, δ, and ε, are splicing variants of TMPOβ/LAP2. All four isoforms share a putative hydrophobic transmembrane domain near their C-terminus via which TMPOβ/LAP2 binds to the NE.[6] However, whereas TMPOβ/LAP2 contains two putative p34cdc2 kinase phosphorylation sites, TMPOδ and TMPOε contain only one such site and TMPOγ lacks both sites, suggesting alternative regulating roles to these isoforms at the NE. TMPOζ, the shortest isoform, lacks both the transmembrane domain of the TMPOβ/LAP2-type isoforms and the NLS of TMPOα.

Monoclonal antibody reacting specifically with LAP2 (TMPO) is a useful tool to study the expression pattern and localization of LAP2/TMPO, in normal cell activity and in apoptosis.

Reagent
Monoclonal Anti-LAP2 (TMPO) is supplied as an approximately 2 mg/ml 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 hazardous and safe handling practices.

Storage/Stability
For continuous use, 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 dilution samples should be discarded if not used within 12 hours.

Product Profile
A working concentration of 2-4 μg/ml is determined by immunoblotting, using a whole extract of cultured human chronic myelogenous leukemia K562 cells.

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

References

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