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Product Information

Monoclonal Anti-Tom22–Atto 488
Clone 1C9-2
produced in mouse, purified immunoglobulin

Catalog Number **T4327**

Product Description

Monoclonal Anti-Tom22 is derived from the 1C9-2 hybridoma (mouse IgG1 isotype) produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a membrane fraction from Vero (monkey kidney-derived) cells.[1] The conjugate is prepared by conjugation of the antibody, purified from ascites fluid, to Atto 488-NHS (λ_{ex} 500 nm; λ_{em} 522 nm), Catalog Number 41698, and the conjugate is purified by gel filtration to remove unbound Atto 488-NHS fluorophore.

Monoclonal Anti-Tom22–Atto 488 conjugate reacts specifically with human Tom22 (GeneID: 56993). Applications include the detection and localization of Tom22 by direct immunofluorescence. Reactivity of Monoclonal Anti-Tom22 has been observed with human and monkey, but not with rodent Tom22.[1]

Many nucleus-encoded mitochondrial proteins are initially synthesized by cytosolic ribosomes as larger pre-proteins with NH_2-terminal pre-sequences, which function as mitochondrial targeting and import signals. The pre-proteins are then targeted to the mitochondria and imported into the organelle. An important step in this process is the interaction of the pre-proteins with the outer surface of the mitochondria. Mitochondria have evolutionarily conserved pre-protein import machinery, located in the outer and inner membranes: the TOM (translocase of outer membrane) and TIM (translocase of inner membrane) complexes, respectively. The fundamental mechanisms of mitochondrial protein import seem to be conserved from lower eukaryotes to mammals.[2] Subtle variations do exist, however, among different species.[1,3] The most studied is the *Saccharomyces cerevisiae* TOM complex. It is composed of at least nine proteins: Tom71, -70, -40, -37, -22, -20, -7, -6, and -5. Tom70, -37, -22, and –20 function as import receptors. Tom71 has strong similarity to Tom70 and is weakly associated with the TOM complex. Tom40 is deeply embedded in the outer membrane in a predicted β-barrel structure and functions as the central component of the translocation channel. Tom6 and Tom7 modulate the dynamics of the TOM channel. Tom5 is tightly associated with Tom40 and represents the connecting link between import receptors and the translocation channel. Thus, Tom40, Tom22, and three smaller Tom proteins form the general pre-protein import pore (the TOM core complex) of ~400 kDa.[1] Studies revealed that Tom22 (22 kDa, also known as 1C9-2[1]) not only functions as the import receptor, but also regulates the organization of the multi-subunit pre-protein translocase TOM complex. The multi-domain protein Tom22 not only functions as a receptor and trans-binding site for pre-proteins, but it also organizes the interaction between the channel and receptor sub complexes at two levels. It is predicted to form a β-sheet or turn structure and serve as a docking point for the peripheral receptors Tom20 and Tom70.

Tom22 has a negatively charged N-terminal region exposed to the cytosol, a putative transmembrane region, and a C-terminal intermembrane space region with little negative charge.[3] The following functions can be assigned to the three domains of Tom22: the cytosolic N-terminal domain plays a dual role, specifically important for pre-sequence binding; the intermembrane space domain provides a trans-binding site for pre-sequences, and the single membrane anchor of Tom22 is crucial for the integrity of the GIP (general import gene) complex. In the absence of this membrane anchor, the GIP complex dissociates into small core complexes containing a dimer of Tom40 and the three small Toms. A pre-protein is stably arrested and accumulated in the GIP complex by Tom40 and Tom22.[4] Such a 100 kDa core complex probably contains a single channel that retains the basic channel properties but is already open in the absence of pre-proteins. In contrast, in the presence of Tom22, the wild-type GIP complex contains tightly regulated channels (probably three channels). Tom22 apparently

represents a component of the machinery that controls the gate.[5] The cytosolic domains of Tom22 and Tom20 are believed to form the major part of a *cis*-site, which mediates the import of all pre-proteins known to use the general import machinery of mitochondria. The pre-protein is then routed through the Tom complex translocation channel and transferred to a *trans*-site on the intermembrane space (IMS) side of the outer membrane. The inter-membrane space-exposed segment of Tom40 and the C-terminal tail of Tom22 may contribute to the *trans*-site. Matrix-targeted proteins are further transferred to the matrix through import machinery in the inner membrane.[6,7] The TOM complex of mammalian mitochondria resembles the fungal Tom complex, but is distinct from the plant TOM system. Thus, while unique components of the mammalian mitochondrial import system have been identified (e.g. TOM34 and metaxin),[1] Tom22, and Tom37 have not been identified in plant mitochondria.[2] Primates have a 19-20% sequence identity to fungal Tom22, while rat Tom22 has a 93.6% identity to human Tom22.[1]

Monoclonal antibodies reacting specifically with Tom22 are useful as a marker for mitochondria and as a tool to study the role of Tom22 in protein translocation across the mitochondria outer and inner membranes.

Reagent
Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: 1.5-3.0 mg/mL
Molar Ratio (F/P): 2-9

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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 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.

Note: Store product protected from light.

Product Profile
Direct Immunofluorescence: a working antibody concentration of 1-2 µg/mL is recommended using human HeLa cells.

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

References

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