

## Product Information

# Anti-TOM22 Antibody, Mouse Monoclonal

Clone 1C9-2, Purified from Hybridoma Cell Culture

**T6319**

## Product Description

Monoclonal Anti-Tom22 (mouse IgG1 isotype) is derived from the 1C9-2 hybridoma 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.<sup>1</sup> The isotype is determined using ImmunoType™ Kit (product code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (product code ISO-2).

Monoclonal Anti-Tom22 reacts specifically with Tom22. The product is useful in immunoblotting (22 kDa)<sup>1</sup> and immunocytochemistry<sup>1</sup> (3.7% paraformaldehyde-0.5% Triton X-100 or 3.7% formaldehyde-0.1% Triton X-100<sup>1</sup> fixation). Reactivity has been observed with human and monkey but not with rodent Tom22.<sup>1</sup>

Many nucleus-encoded mitochondrial proteins are initially synthesized by cytosolic ribosomes as larger preproteins with NH2-terminal pre-sequences, which function as mitochondrial targeting and import signals. The preproteins are then targeted to the mitochondria and imported into the organelle. An important step in this process is the interaction of the preproteins with the outer surface of the mitochondria. Mitochondria have an evolutionarily conserved preprotein 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.<sup>2</sup> Subtle variations do exist, however, among different species.<sup>1,3</sup> 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  $\beta$ -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 preprotein import pore (the TOM core complex) of approximately 400 kDa.<sup>1</sup>

Studies revealed that Tom22 (22 kDa, also known as 1C9-2<sup>1</sup>) not only functions as the import receptor, but also regulates the organization of the multi subunit preprotein translocase TOM complex. The multidomain 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 subcomplexes at two levels. It is predicted to form a  $\beta$ -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.<sup>3</sup> 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.
- 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 preprotein is stably arrested and accumulated in the GIP complex by Tom40 and Tom22.<sup>4</sup> 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 preproteins. In contrast, in the presence of Tom22, the wild-type GIP complex contains tightly regulated channels (probably three channels).

Tom22 reportedly represents a component of the machinery that controls the gate.<sup>5</sup> The cytosolic domains of Tom22 and Tom20 are believed to form a major part of a cis site, which mediates the import of all preproteins known to use the general import machinery of mitochondria. The preprotein 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.<sup>6,7</sup>

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)<sup>1</sup>, Tom22, and Tom37 have not been identified in plant mitochondria.<sup>2</sup> Primates have a 19-20% sequence identity to fungal Tom22, while rat Tom22 has a 93.6% identity to human Tom22.<sup>1</sup>

Monoclonal antibody reacting specifically with Tom22 is a useful tool to study the role of Tom22 in protein translocation across the mitochondria outer and inner membranes.

## Reagent

Monoclonal anti-TOM22 is supplied as an approximately 1 mg/mL solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

## Precautions and Disclaimer

Due to the sodium azide content, a safety data sheet (SDS) for this product has been sent to the attention of the safety officer of your institution. Consult the SDS for information regarding hazards 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 0.5-1 µg/mL is determined by immunoblotting, using a whole extract of cultured human lymphoma Raji cells.

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

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## References

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3. Yano, M., et al., Mol. Cell. Biol., 20, 7205-7213 (2000).
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7. Kanamori, T., et al., Proc. Natl. Acad. Sci. USA., 96, 3634-3639 (1999).

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