

MONOCLONAL ANTIBODY **CTLA-4 (CD152)**

Cat. No.	Form	Quantity	Presentation
2070	Purified	0.2 mg	Freeze-dried
2282	PE	100 tests	Liquid 2 mL

Clone BNI3

Isotype IgG2a (mouse)

Immunogen Human CTLA-4 / human IgG heavy chain fusion protein (CTLA-4/Ig).

Hybridoma P3/X63 - Ag8.653 myeloma cell x Balb/c spleen cell

Specificity The "cytolytic T-lymphocyte-associated antigen 4" (CTLA-4) is a member of the Ig superfamily (1) and its extracellular domain exhibits a single Ig V-like region. It was originally identified as a fourth cDNA product, during a search for genes that were thought to be specifically expressed in cytotoxic T lymphocytes (1).

CTLA-4 has a molecular weight close to 45 kDa (2). It is expressed as a disulfide-linked homodimer (2, 3), but may also be expressed as a monomer (4).

CTLA-4 belongs to the CD28 receptor family (4, 5). CTLA-4 and CD28 show similar (and evolutionarily conserved) cytoplasmic tails, and 31% homology at the amino acid level (4). The gene for CTLA-4 maps to the same chromosomal band (2q33, in humans) as the CD28 gene (5).

Interestingly, both molecules interact with the same ligands, namely B7.1 (CD80 antigen) and B7.2 (CD86 antigen) (6). However, the avidity of interaction of B7.1 and B7.2 for CD28 is about 20-fold lower than for CTLA-4 (2, 7).

In contrast to CD28, which is expressed on 80% of human peripheral blood T cells, CTLA-4 is not constitutively expressed on the surface of T cells, even if the molecule is often present in the cytoplasm. It is lately and transiently detectable on the membrane, after T-cell activation. The surface expression of CTLA-4 peaks 3 days after *in vitro* activation (8) and drops to undetectable levels 4 days later. Moreover, only low levels of CTLA-4 are present at the surface of the activated cells at the maximum of expression (9).

Originally, CD28 and CTLA-4 were both considered as costimulatory effectors in T-cell activation (8, 10). However, recent studies highlight an inhibitory role for CTLA-4 (11): It was shown to down-regulate CD28-induced T-cell activation (7), and it is also suspected to be involved in induction of apoptosis of human T cells (12). In the absence of CTLA-4 expression, activated T cells can spontaneously proliferate in mice (13).

Additionally, CTLA-4 was recently reported to be expressed on human B cells at even higher levels than on activated T cells, when cultured with activated T cells, using mixed CD2 activating monoclonal antibodies (14).

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MA003

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Immunohistological studies on human lymphoid tissues have shown that the BNI3 monoclonal antibody reacts exclusively with $\alpha\beta^+$ T cells (15).

In germinal centers, CD4⁺ helper T cells are mostly CTLA-4⁺ (70-90%) (15). The intracellular distribution of CTLA-4 is primarily concentrated in cytoplasmic vesicles (16).

Applications

Studies of the immune response involving interactions between immunocompetent cells.

Research studies of the "co-stimulatory" role of CTLA-4 molecule during T-cell and/or B-cell activation and regulation.

Research studies by microscopy or flow cytometry, after *in vitro* activation of T cells with selected stimuli (Concanavalin A, or solid-phase fixed-CD3 + CD28 mAbs stimulation).

Research studies on CTLA-4 expression on B cells.

Research studies on CTLA-4 expression by immunohistochemistry only on frozen tissue sections.

Buffer

Freeze-dried form: 1 mg/mL bovine serum albumin in phosphate-buffered saline, pH 7.2.

Liquid form: 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

Conjugation

Phycoerythrin: R-Phycoerythrin conjugated (1 mole of phycoerythrin/mole of IgG).
Excitation wavelength : 488 nm,
Maximum emission wavelength : 575 nm,
Main emission color: orange-red.

Reconstitution and Storage

The freeze-dried form may be stored at 2-8°C until the expiration date. Reconstitute with 1 mL of distilled water. No preservative has been added. The reconstituted form may be stored at -20°C until the expiration date. Aliquotting is suggested to avoid multiple freeze-thaw cycles. The addition of sodium azide at 0.1% (w/v) is recommended for storage of the reconstituted form for up to one month at 2-8°C.

The conjugated forms should not be frozen and should be stored in the dark at 2 - 8°C until the expiration date stated on the vial label.

Recommended Procedures

Fluorescence microscopy or flow cytometry

Liquid form: 20 μ L / 5 x 10⁵ cells / test or 100 μ L of whole blood

Freeze-dried: 2 μ g / 5 x 10⁵ cells / test or 100 μ L of whole blood

CTLA-4 molecule can be detected at the intracellular level using BNI.3 PE conjugated monoclonal antibody after permeabilization with a saponine-based procedure. The sample volume should be adjusted to the permeabilization procedure used.

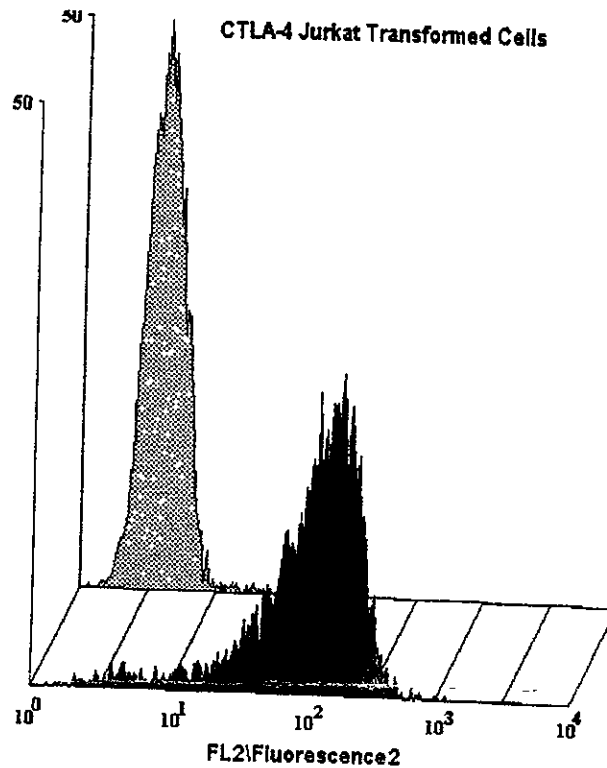
Cytospin or immunohistochemistry on frozen tissue sections

Recommended form: Freeze-dried

Working concentration: 5-50 μ g/mL

Results

The flow cytometric analyzed histogram below illustrates the direct labeling of transfected Jurkat cells with full length human CTLA-4.



Cells are labelled with PE conjugated BNI.3 monoclonal antibody (Cat. No. 2282).

The grey profile represents the control experiment realized with a PE conjugated IgG2a isotypic control (Cat. No. 0671) instead of BNI.3.

References

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