

**PN IM2549**      **Monoclonal Antibody**      **TcR $\zeta$**

<b>Form</b>	Unconjugated	<b>Clone</b>	2H2D9 (TIA-2)
<b>Quantity</b>	0.2 mg	<b>Isotype</b>	IgG1, $\kappa$
<b>Presentation</b>	Freeze-dried	<b>Species</b>	Mouse
<b>Purity</b>	Purified IgG		

For Research Use Only. Not for use in diagnostic procedures.

**SPECIFICITY**

The T cell antigen receptor (TcR) is composed by the clonotypic heterodimer TcR $\alpha/\beta$  or TcR $\gamma/\delta$ , associated with the multisubunit CD3 complex. This CD3 complex brings together five different chains:  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$  and  $\eta$ . CD3 complex consolidation is made through a group of two invariant dimers, CD3 $\gamma/\epsilon$  and CD3 $\delta/\epsilon$ , associated with a variable dimer of CD3 $\zeta$  family molecules. These variable dimers are  $\zeta$  homodimers, or  $\zeta$ - $\eta$ , or  $\zeta$ -Fc $\epsilon$ R $\gamma$  chain heterodimers (Fc $\epsilon$ R $\gamma$  chain being the  $\gamma$  chain of the high-affinity receptor complex for IgE, also present in the low-affinity receptor complex for IgG), or a dimer of the Fc $\epsilon$ R $\gamma$  chain. The  $\zeta$  chain belongs to a family of structurally and functionally related molecules that includes  $\zeta$ ,  $\eta$  and Fc $\epsilon$ R $\gamma$ . The CD3 $\zeta$  and  $\eta$  chains result from the alternative splicing of a single gene designated CD3  $\zeta/\eta$  (1, 2). The  $\zeta$  chain is a 16-kDa integral membrane protein with a short extracellular domain of 9 amino acids and a cytoplasmic tail of 113 amino acids (3). Its expression is restricted to T lymphocytes, TcR-negative thymocytes and NK cells (4, 5, 6).

The  $\zeta$  chain is involved in the transduction of signals after the TcR engages its ligand, through the activation of motifs in the cytoplasmic region (immunoreceptor tyrosine-based activation motifs: ITAMs) of the molecule.

The  $\zeta$  chain is also involved in the regulation of the assembly and intracellular transport of the TcR-CD3 complex (1, 2).

On T lymphocytes, the  $\zeta$  subunit is expressed as a disulfide-linked homodimer with a molecular weight (Mr) of 32 kDa or as disulfide-linked heterodimer  $\zeta$ - $\eta$ . On NK cells, the  $\zeta$  chain is expressed as a disulfide-linked homodimer ( $\zeta$ - $\zeta$ ) or physically associated with the CD16 molecule (4). As on T lymphocytes, the  $\zeta$  chain constitutes a signal-transducing subunit in human NK cells (5). The level of expression of CD3  $\zeta$  may correlate with the functional state (e.g. activation and maturation) of  $\zeta$ -expressing cells (3).

2H2D9 (TIA-2) monoclonal antibody recognizes the cytoplasmic domain of the  $\zeta$  subunit. A digitonin-based permeabilization procedure is required to reach the intracellular targeted antigen (7, 8).

2H2D9 (TIA-2) monoclonal antibody was assigned to the CD3 $\zeta$  cluster during the 6th International Workshop on Human Leucocyte Differentiation Antigens in Kobe (Japan) in 1996 (3).

**APPLICATIONS**

- Flow cytometry.  
Since 2H2D9 (TIA-2) monoclonal antibody recognizes the cytoplasmic domain of the  $\zeta$  subunit, a digitonin-based permeabilization procedure is required to reach the intracellular targeted epitope (7, 8).
- Immunoblotting (4).
- Immunoprecipitation (4).
- Research studies of  $\zeta$  chain role during T-cell and NK-cell development.
- Research studies of T-cell and NK-cell signal transduction pathways involving  $\zeta$  chain.

**BUFFER**

1 mg/mL bovine serum albumin in phosphate-buffered saline.

**STORAGE CONDITIONS AND STABILITY**

This freeze-dried form may be stored at 2–8°C until the expiration date stated on the vial label. No preservative has been added.

**REAGENT PREPARATION**

Depending of usage, reconstitute with 1 mL of distilled water, with or without 0.1% sodium azide (w/v). The reconstituted form including 0.1% sodium azide may be stored for up to one month at 2–8°C. The reconstituted form without sodium azide can be stored at -20°C or less, until the expiration date stated on the vial label. Aliquotting is recommended to avoid multiple freezing / thawing cycles.

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Quantity	0.2 mg	Isotype	IgG1, $\kappa$
Presentation	Freeze-dried	Species	Mouse
Purity	Purified IgG		

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## PROCEDURE

Flow cytometry.

Assay volume: 10  $\mu$ L of the reconstituted purified antibody per  $5 \times 10^5$  cells per test, or per 100  $\mu$ L whole blood.

## SELECTED RESEARCH REFERENCES

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4. [2836] Anderson, P., Caligiuri, M., O'Brien, C., Manley, T., Ritz, J., Schlossman, S.F., "Fc $\gamma$  receptor type III (CD16) is included in the  $\zeta$  NK receptor complex expressed by human natural killer cells", 1990, *Proc. Natl. Acad. Sci. USA*, 87, 2274-2278.
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6. [4260] Moingeon, P., Stebbins, C.C., D'Adamio, L., Lucich, J., Reinherz, E.L., "Human natural killer cells and mature T lymphocytes express identical CD3 $\zeta$  subunits as defined by cDNA cloning and sequence analysis", 1990, *Eur. J. Immunol.*, 20, 1741-1745.
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