PN IM3169 TcRζ-PE (2H2D9)

100 tests
20 µL / test

IOTest®
Conjugated Antibodies

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

SPECIFICITY

The T cell antigen receptor (TcR) is composed by the clonotypic heterodimer TcRα/β or TcRγ/δ, associated with the multisubunit CD3 complex. This CD3 complex brings together five different chains: γ, δ, ε, ζ and η. CD3 complex consolidation is made through a group of two invariant dimers, CD3γ/ε and CD3δ/ε, associated with a variable dimer of CD3ζ family molecules. These variable dimers are ζ homodimers, or ζ-η, or ζ-FcεRγ chain, or a dimer of the FcεRγ chain (FcεRγ chain being the γ chain of the high-affinity receptor complex for IgE, also present in the low-affinity receptor complex for IgG). The ζ chain belongs to a family of structurally and functionally related molecules that includes ζ, η and FcεRγ. The CD3ζ and η chains result from the alternative splicing of a single gene designated CD3 ζ/η (1, 2). The ζ 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 ζ 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 ζ chain is also involved in the regulation of the assembly and intracellular transport of the TcR-CD3 complex (1, 2).
On T lymphocytes, the ζ subunit is expressed as a disulfide-linked homodimer with a molecular weight (Mr) of 32 kDa or as disulfide-linked heterodimer ζ-η. On NK cells, the ζ chain is expressed as a disulfide-linked homodimer (ζ-ζ) or physically associated with the CD16 molecule (4). As on T lymphocytes, the ζ chain constitutes a signal-transducing subunit in human NK cells (5). The level of expression of CD3 ζ may modulate the function (e. g. activation and maturation) of ζ-expressing cells (3).

2H2D9 (TIA-2) monoclonal antibody recognizes the cytoplasmic domain of the ζ 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 CD3ζ cluster during the 6th international workshop on Human Leukocyte Differentiation Antigens in Kobe (Japan) in 1996 (3).

REAGENT

Clone	2H2D9
Isotype	IgG1,κ (mouse)
Immunogen	Digitonin permeabilized human peripheral blood T lymphocytes
Hybridoma	Myeloma NS1 x Balb/c spleen cells
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of Ig. Excitation wavelength: 488 nm Maximum emission wavelength: 575 nm Main emission color: Orange-red
Buffer	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

Flow cytometry.

Since 2H2D9 (TIA-2) monoclonal antibody recognizes the cytoplasmic domain of the ζ subunit, a permeabilization procedure is required to reach the intracellular targeted antigen (7, 8).

Research studies of ζ chain role during T-cell and NK-cell development.
Research studies of T-cell and NK-cell signal transduction pathways involving ζ chain.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2–8°C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20–25°C prior to use.

PROCEDURE

A- For human cell lines or purified cells, such as peripheral blood lymphocytes (PBL), the following digitonin-based procedure is recommended.

Cytoplasmic TcRζ-PE (PN IM3169) staining combined with membrane staining using conjugated antibodies.

1. Deliver 100 µL of a 5 x 10^6 cells / mL suspension to 12 x 75 mm siliconized glass tubes.
2. Add 20 µL (or 10 µL if recommended on the procedure) of the membrane-specific conjugated antibody (e. g. CD3-FITC, PN IM1281) and incubate 15 min. at room temperature (RT) in the dark.
3. Add 3 mL of PBS / NaN3 (*), centrifuge at 200 x g for 5 min. at RT and discard the supernatant.
4. Add 100 µL of PBS / 0.25% formaldehyde (v/v), vortex gently, and incubate 10 min. at RT in the dark.
5. Repeat step 3 (washing).
6. Add 100 µL of digitonin at 100 µg/mL (**) in PBSF (***), gently resuspend the cells.
7. Add 20 µL of anti-TcRζ-PE, vortex gently and incubate for 10 min. on ice in the dark.
8. Repeat step 3 (washing).
9. Gently resuspend the cells with 0.5 to 1.0 mL of PBS / 0.25% formaldehyde (v/v).

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10. Store at 2–8°C in the dark until acquisition on the flow cytometer.
    (*) PBS containing 0.01% NaN_3 (v/w)
    (**) Preparation of stock working solution of 25 mg/mL of digitonin in PBS:
    Dissolve 250 mg digitonin (Fluka, product number 37006 "water soluble") in 10 mL of PBS. Place in hot water bath (80°C) for 10 minutes. Solution should be clear. Allow to cool to room temperature. Filter solution through 0.80 µm filter unit and then refilter through 0.22 µm filter unit. Store at 2–8°C (3 month shelf-life).
    (***) PBS with 2.5% fetal calf serum (v/v) and 0.01% NaN_3 (v/w).

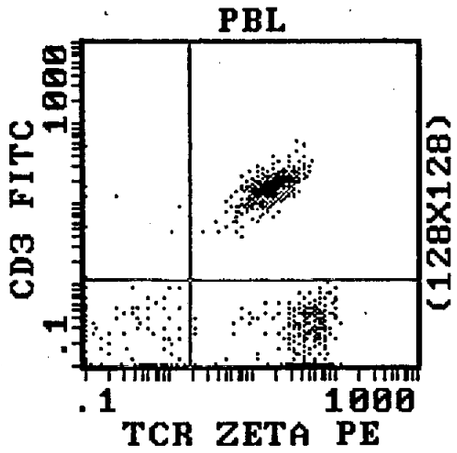
B- For fixation and permeabilization of whole blood samples a formaldehyde / saponin-based protocol, using low formaldehyde (fixative) concentration (i. e. 0.5%, final concentration) is strongly recommended.

EXAMPLE DATA

The graph below is a biparametric representation (Fluorescence Intensity 1 versus Fluorescence Intensity 2) of digitonin-permeabilized normal peripheral blood lymphocytes (PBL). Intracellular staining is performed with TcRζ-PE (PN IM3169) combined with CD3-FITC (PN IM1281) cell surface staining. Gate is on PBL events.

*Upper-left quadrant contains potential CD3-positive, TcRζ-negative events.
*Upper-right quadrant contains double positive CD3+TcRζ+, i.e.: TcRζ expressing T lymphocytes.
*Lower-left quadrant contains double negative events.
*Lower-right quadrant contains CD3-negative, TcRζ-positive PBL and includes NK cells.

Acquisition is with a COULTER® EPICS® XL™ flow cytometer. Analysis is with the XL SYSTEM II™ software.



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