

PN IM3293**TIA-1-PE****(2G9)****100 tests****20 µL / test****SPECIMEN
POUR INFORMATION**

For Research Use Only. Not For Use In Diagnostic Procedures.

SPECIFICITY

TIA-1 is a 17 kDa cytoplasmic granule-associated protein, expressed in cells possessing cytolytic potential (1, 2), and recently designated as GMP-17, for granule membrane protein of 17 kDa (3). The GMP-17 / TIA-1 molecule could be involved in the molecular cascade of signaling Fas (CD95)-mediated apoptosis (2 - 4).

Within hematopoietic cell lines, the "TIA-1" monoclonal antibody (clone 2G9) reacts with about 90% of CD16⁺, 50-60% of CD8⁺, and less than 10% of CD4⁺ normal peripheral blood lymphocytes (5). It reacts with almost all monocytes and granulocytes (3). This antibody also reacts with CD4⁺ activated T-cell clones, activated NK cell clones, and Con A-activated thymocytes, but not with B lymphocytes or B-cell lines (1, 5).

The 2G9 monoclonal antibody (mAb) is a useful reagent for detecting cytolytic effector cells in tissue infiltrates (3, 6, 7). In research studies using the 2G9 mAb with other markers of activated cytotoxic cells (such as granzyme B), a high percentage of activated CTLs was detected in biopsy material of Hodgkin's disease patients, demonstrating that GMP-17 / TIA-1 represents a strong indicator for an unfavorable clinical outcome (8). This antibody was also used in research studies of lymphocyte subsets undergoing apoptosis in lymph nodes of HIV-1⁺ patients (9 - 11).

The 2G9 mAb was evaluated during the 5th International Workshop on Human Leucocyte Differentiation Antigens, in the section of monoclonal antibodies reactive with intracellular antigens (6).

REAGENT

Clone	2G9 (2G9A10F5 or TIA-1)
Isotype	IgG1, κ
Immunogen	Digitonin-permeabilized human peripheral blood T lymphocytes
Hybridoma	NS/1-Ag4 x Balb/c
Species	Mouse
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	R-phycoerythrin (PE) is conjugated at 0.5 - 1.5 moles 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.

STATEMENT OF WARNINGS

- 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.
- 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.
- Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
- Do not use antibody beyond the expiration date on the label.
- Do not expose reagents to strong light during storage or incubation.
- 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**Flow Cytometry.**

Permeabilization procedure using IntraPrep™ Permeabilization Reagent (PN IM2388, PN IM2389).

Described is the procedure for combined membrane / cytoplasmic staining using a membrane-specific conjugated monoclonal antibody, followed by intracytoplasmic staining with conjugated antibodies:

- Dispense 50 µL of whole blood (or 5 x 10⁶ white blood cells) into 2 tubes for each sample:
 - One membrane and cytoplasmic staining test tube = assay tube
 - One membrane and cytoplasmic isotypic control tube = control tube
- Add 20 µL of membrane-specific conjugated monoclonal antibody to the assay tubes and 20 µL of appropriate membrane isotypic control to the control tubes.
- Vigorously vortex tube by tube and incubate for 15 minutes at room temperature (18 - 25°C) in the dark.
- Add 100 µL of IntraPrep Reagent 1 to each tube.
- Vigorously vortex tube by tube and incubate for 15 minutes at room temperature in the dark.
- Add 4 mL of PBS, centrifuge for 5 minutes at 300 x g at room temperature and discard supernatant (by aspiration).
- Add 100 µL of IntraPrep Reagent 2 to each tube.
- Let binding occur WITHOUT VORTEXING.
- Incubate for 5 minutes at room temperature WITHOUT VORTEXING.
- Gently agitate (manually), for 1 to 2 seconds.
- Add 20 µL (or 10 µL, depending on the manufacturer recommendations) of intracellular conjugated specific antibody to the assay tubes, and 20 µL (or 10 µL, depending on the manufacturer recommendations) of the appropriate intracellular control reagent (e.g. conjugated isotypic control or conjugated specific antibody used for control) to the control tubes.
- Repeat step 8 (washing).
- Resuspend cells in 500 µL of PBS containing 0.5% formaldehyde and proceed to flow cytometry analysis.
- The specimen should be analyzed within two hours after IntraPrep treatment when stored at room temperature. Otherwise, fixed preparations should be stored at 2 - 8°C in the dark and analyzed within 24 hours.

EXAMPLE DATA

The graphs next page are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of normal human whole blood sample.

Membrane staining is with IOTest CD8-FITC (PN IM0452). Intracellular staining is with TIA-1-PE (PN IM3293). The gating is on the lymphocytes. Permeabilization is with IntraPrep Permeabilization Reagent (PN IM2388).

Figure 1:

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

3293EX220299 Vers.1 / 18/03/99 AC-99069

1/2



PN IM3293 TIA-1-PE

(2G9)

**100 tests
20 µL / test**

**SPECIMEN
POUR INFORMATION**

For Research Use Only. Not For Use In Diagnostic Procedures.

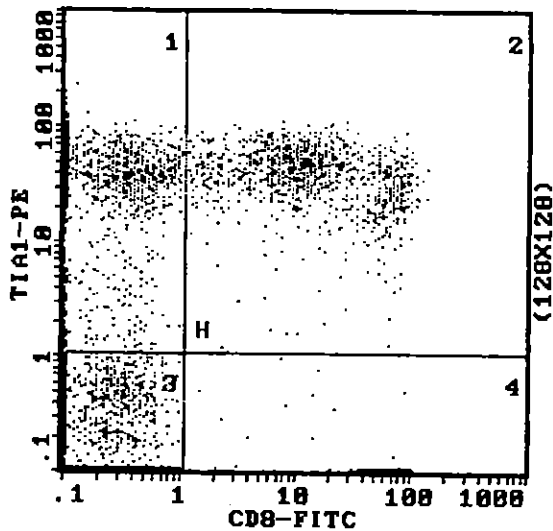
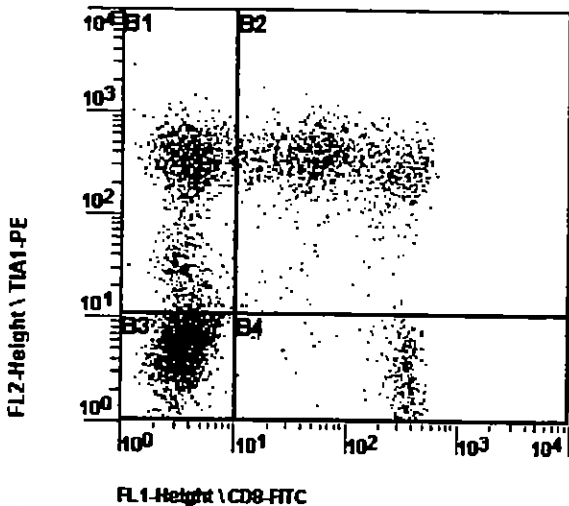


Figure 2:

Acquisition with a Becton Dickinson FACScan™ flow cytometer equipped with LYSYS II™ software. Analysis with EXPO™ (v. 2) Cytometer software (Coulter PN 6605334).

(1):TIA1001 FL1-H/FL2-H A



SELECTED RESEARCH REFERENCES

- [1127] Anderson, P., Nagler-Anderson, C., Obrien, C., Levine, H., Watkins, S., Slayter, H.S., Blue, M-L., Schlossman, S.F., "A monoclonal antibody reactive with a 17 kDa cytoplasmic granule associated protein defines a subpopulation of CD8+ T lymphocytes", 1990, J. Immunol., 2, 144, 574.
- [2585] Tian, Q., Streuli, M., Saito, H., Schlossman, S.F., Anderson, P., "A polyderylate binding protein localized to the granules of cytolytic lymphocytes induces DNA fragmentation in target cells", 1991, Cell, 67, 629-639.
- [5123] Meehan, S.M., McCluskey, R.T., Pascual, M., Preffer, F.I., Anderson, P., Schlossman, S.F., Colvin, R.B., "Cytotoxicity and apoptosis in human renal allografts: Identification, distribution, and quantitation of cells with a cytotoxic granule protein GMP-17 (TIA-1) and cells with fragmented nuclear DNA", 1997, Lab. Invest., 78, 639-649.
- [417] Tian, Q., Taupin, J.L., Elledge, S., Robertson, M., Anderson, P., "Fas-activated serine/threonine kinase (FAST) phosphorylates TIA-1 during Fas-mediated apoptosis", 1995, J. Exp. Med., 182, 865-874.
- [2578] Francis, C., Connelly, M.C., "Rapid single-step method for flow cytometric detection of surface and intracellular antigens using whole blood", 1996, Cytometry, 25, 58-70.
- [2690] Anderson, P., "mAb reactive with lymphocyte-restricted intracellular antigens", 1995, Leucocyte Typing V, White Cell Differentiation Antigens. Schlossman, S.F., et al., Eds., Oxford University Press, 325-327.
- [2586] Russell, G.J., Nagler-Anderson, C., Anderson, P., Bhan, A.K., "Cytotoxic potential of intraepithelial lymphocytes (IELs)", 1983, Am. J. Pathol., 2, 143, 350-354.
- [2577] Oudejans, J.J., Jiwa, N.M., Kummer, J.A., Ossenkoppele, G.J., Van Heerde, P., Baars, J.W., Kuin, Ph.M., Kuin-Nelemans, J.C., Van Diest, P.J., Meddendorp, J.M., Meijer, C.J.L.M., "Activated cytotoxic T cells as prognostic marker in Hodgkin's disease", 1997, Blood, 4, 89, 1376-1382.
- [2582] Bofill, M., Gombert, W., Borthwick, N.J., Akbar, A.N., McLaughlin, J.E., Lee, C.A., Johnson, M.A., Pinching, A.J., Janossy, G., "Presence of CD3+, CD8+, Bcl-2low lymphocytes undergoing apoptosis and activated macrophages in lymph nodes of HIV-1+ patients", 1995, Am. J. Pathol., 146, 1542-1555.
- [2583] Boudet, F., Lecoq, H., Gougeon, M.L., "Apoptosis associated with ex vivo down-regulation of Bcl-2 and up-regulation of fas in potential cytotoxic CD8+ T lymphocytes during HIV infection", 1996, J. Immunol., 158, 2282-2293.
- [2584] Tenner-Racz, K., Racz, P., Thomé, C., Meyer, C.G., Anderson, P.J., Schlossman, S.F., Letvin, N.L., "Cytotoxic effector cell granules recognized by the monoclonal antibody TIA-1 are present in CD8+ lymphocytes in lymph nodes of human immunodeficiency Virus-1 infected patients", 1993, Am. J. Pathol., 142, 1750-1758.