ZAP70-PE

Ref: CYT-ZAP70PE3

For Research Use Only

INTENDED USE

ZAP70-PE is a monoclonal antibody (MAb) labelled with R-phycoerythrin (PE) designed for flow cytometry use as a direct immunofluorescence reagent in the identification and enumeration of ZAP70 antigen-expressing cells.

SUMMARY AND EXPLANATION

Flow Cytometry (FC) is a powerful tool in analytical and quantitative characterization of cells which provides rapid and multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis. Flow cytometry is performed on cell suspension after incubating it with fluorescent-labelled antibodies directed against specific cellular proteins. Positive cells relative fluorescence intensity indicates the amount of antibody bonded to specific cell sites providing information about antigen expression.

ZAP70 is a tyrosine kinase of the Syk family that plays a critical role in triggering the signalling cascade in response to T-cell receptor stimulation [(1)]. It is primarily expressed in T cells and natural killer (NK) cells [(1-6)], but is also detectable in mast cells and basophils [(2)]. ZAP70 is also expressed in normal pro/pre B cells but not in normal mature B cells [(4)].

ZAP70 is expressed in B cells in a subset of chronic lymphocytic leukemias (CLL) closely associated with an unmutated configuration of the immunoglobulin heavy-chain variable region (IgVH) genes [(1-6)]. The precise functional significance of ZAP70 in this subset of CLL B cell is unknown. Some data indicates that expression of ZAP70 in CLL allows more effective IgM signaling in CLL B cells, which could explain the disease progress [(5)]. Also, some other B-cell neoplasms [(1-6)] and T-cell neoplasms express ZAP-70 [(6)].

PROCEDURE PRINCIPLES

Flow cytometry is an innovative technology that can evaluate simultaneously different characteristics for a single cell. Flow cytometers use hydrodynamic focusing to individually present cells to one or more laser beams. As cells are intercepted by light a set detectors recover signals of two different kinds: those generated by dispersed light (FSC/SSC), which mainly reflect cell size and internal complexity, and those related to fluorochromes light emission when cells are labelled. Recovered signals are then amplified by a series of linear and logarithmic amplifiers and converted in electrical signals large enough to be plotted graphically.

Fluorochrome-labelled monoclonal antibodies bind specific antigens, therefore cell populations carrying this antigen will be detected when the reagent is added to a sample and passed through a flow cytometer.

CYT-ZAP70PE3 should be used with a cell permeabilization kit which gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of cells intact.

ZAP70+ is usually expressed as a percentage of total B cells present in a sample. Since every flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION

The purified monoclonal ZAP70 antibody conjugated with R-phycoerythrin (PE) is supplied in phosphate-buffered saline (PBS) containing 0.1% sodium azide.

Clone: 1E7.2.

Isotype: IgG1.

Amount per 1 ml vial: 100 tests (10 µl MAb per determination).

Reagent is considered non-sterile.

STORAGE CONDITIONS

The reagent is stable when stored at 2-8 ºC until expiration date shown on label. The reagent should not be frozen or exposed to direct light during storage or during cell incubation. Reagent vial should be kept dry and once open stored in vertical position to avoid any possible spillage.

WARNINGS AND RECOMMENDATIONS

1. For research use only.
2. This product is supplied ready to use, any modification by dilution or addition of other compounds should be validated by the user.
3. The reagent is stable until its expiry date when properly stored. Do not use it after expiration date shown on label. If product is stored in conditions different from those recommended, such conditions must be validated by the user.
4. Alteration in reagent appearance, such as precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.
5. It contains 0,1% sodium azide (CAS-Nr. 26628-22-8) as a preservative but care should anyway be taken to avoid microbial contamination of reagent and reduce incorrect results that can probably arise from that contamination.
    - Sodium azide (NaN_3) is harmful if swallowed (R22). If swallowed, seek medical advice immediately and show this container or label (S46).
    - Wear suitable protecting clothing (S36).
    - Contact with acids liberates very toxic gas (R32).
    - On disposal, flush with large amounts of water to prevent azide build-up in metal plumbing since explosive conditions may develop.
6. All patient specimens and materials are considered biohazards and should be handled as if capable of transmitting infection [(7)]. Disposal should be made according to the established legal precautions. It is also recommendable the use of appropriate protective gloves and clothing when handling this product. Product use should be made by personnel qualified to perform the described procedures. Avoid sample contact with skin or mucous membranes. Wash immediately with abundant water if skin contact has occurred.
7. Reagent use with incubation times or temperatures different from those recommended may cause erroneous results. Any changes in procedure must be validated by the user.

PROCEDURE

Material included
ZAP70-PE can be used for 100 determinations (10 µl MAb per determination).

Material required but not included
- 488 nm ion argon laser-equipped flow cytometer and appropriate computer hardware and software.
- Test tubes suitable for the used flow cytometer. Usually 6 mL tubes (12x 75 mm) with a rounded bottom are used.
- Automatic pipette (100µL) and tips.
- Micropipette and tips.
- Chronometer.
- Vortex Mixer.
- Isotypic control reagent.
- Cell permeabilization kit.
- Wash buffer (phosphate buffered saline (PBS) containing 0.1% sodium azide).

Preparation
Whole blood sample must be taken aseptically by means of a venipuncture [8, 9] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). The analysis requires 50 µl of the whole blood sample per tube, assuming a normal range of approximately 4 to 10 x 10^3 leucocytes per µl. For samples with a high white blood cell count, dilute samples with PBS to obtain a concentration of cells approximately equal to 1 x 10^4 cells/µl. Store the blood samples at 18-22ºC until they are to be tested. It is advisable to test blood samples within the 24 hours after their extraction. Hemolyzed samples or samples with suspended cell aggregates should be rejected.

1. Transfer 50 µl of the cell suspension to be analyzed (whole blood, bone marrow or mononuclear cells) to a test tube.
2. In case of use a staining protocol of cell surface antigens and intracellular antigens, add the appropriate volume of antibodies to surface staining. Mix gently by using a vortex mixer and incubate at room temperature for 15 minutes.
3. Add 100 µl of Fixation Reagent. Mix gently to ensure that the cells are in suspension.
4. Incubate at room temperature for 15 minutes.
5. Add 2 ml PBS and mix gently by using a vortex mixer.
6. Centrifuge at 540g for 5 minutes.
7. Aspirate the supernatant, leaving approximately 50 µl of fluid.
8. Mix thoroughly by using a vortex mixer to ensure that the cells are in suspension and add 100 µL of Permebilization Reagent. Add 10 µL of CYT-ZAP70PE3. Mix gently
   Use a non-reactive monoclonal antibody of the same isotype, and conjugated with the same fluorochrome, as a negative control.
9. Incubate in the dark at room temperature for 15 minutes.
10. Add 2 ml PBS and mix gently by using a vortex mixer.
11. Centrifuge at 540g for 5 minutes.
12. Aspirate the supernatant, leaving approximately 50 µl of fluid.
13. Resuspend the cell pellet in 300 µl PBS.
14. Acquire on a flow cytometer within the first three hours of finishing the sample preparation.

Flow cytometry analysis
Confirm that the cytometer is correctly aligned and standardised for light dispersion and fluorescent intensity. Compensation should be set following cytometer manufacturer instructions.
Results are commonly reported as a percentage of sample total B lymphocyte count present.

LIMITATIONS
- Blood samples should be stored at 18-22ºC and tested within 24 hours after they were obtained.
- It is advisable to acquire stained samples as soon as possible to optimise results. Non-viable cells may show unspecific staining. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction and targeted population cell loss.
- When using whole blood procedures some red blood cells may not lyse, for instance if there are nucleated red blood cells or if abnormal protein concentration and haemoglobinopathies are observed. This may cause misleading results since unlysed red blood cells are counted as leucocytes.
- Results obtained by flow cytometry may be erroneous if cytometer laser is misaligned or if gates are incorrectly set.
- Each laboratory should establish a normal range for ZAP70+ cells using its own test conditions.
- Certain patients may present special problems due to altered or very low number of a certain cellular population.
- Cells separated from whole blood by means of density gradients may not have the same relative concentration as in whole blood. This may be relatively insignificant in individuals with normal white blood cell counts. In leucopenic patients, the selective loss of specific subsets may affect determination accuracy.
- Knowledge of antigen normal expression pattern and its relation to other relevant antigens is paramount to carry out an adequate analysis [1-6].
- Abnormal states of health are not always represented by abnormal percentages of certain leucocyte populations. An individual in an abnormal state of health may show the same leucocyte percentage as a healthy person.

QUALITY CONTROL
- Pipettes precision and cytometer calibration should be verified to obtain optimal results.
- In multicolour panels, fluorochromes emit in wavelengths that can show certain spectral overlap which must be corrected by electronic compensation. Optimal compensation levels can be established by analysing cells from healthy individuals stained with mutually exclusive monoclonal antibodies conjugated with appropriate fluorochromes.
- Non-specific binding of the antibody can be evaluated using an appropriated isotype control tube.

REFERENCES
1. Carreras J, Villamor N, Colomo L, Moreno C, Cajal S, Crespo M, et al. Immunohistochemical analysis of ZAP70 expression in B-cell lymphoid neoplasms. J Pathol 2005;205;507-13 (2005).

2. Wiestner A, Rosenwald A, Barry TS, Wright G, Davis RE, Henrickson SE, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 101:4944-51 (2003).
3. Keating MJ, Chiorazzi N, Messmer B, Damle RN, Allen SL, Rai KR, et al. Biology and Treatment of chronic lymphocytic leukemia. Hematology 153-75 (2003).
4. Crespo M, Villamor N, Giné E, Muntañola A, Colomer D, Marafioti T, et al. ZAP-70 expression in normal pro/pre B cells, mature B cells, and in B cell acute lymphoblastic leukemia. Clin. Cancer Res. 12:726-34 (2006).
5. Chen L, Apgar J, Huynh L, Dicker F, Giago-McGahan T, Rassenti L, et al. ZAP-70 directly enhances IgM signaling in chronic lymphocytic leukemia. Blood 105:2036-41 (2005).
6. Sup SJ, Domiati-Saad R, Kelley TW, Steinle R, Zhao X, Hsi ED. ZAP-70 expression in B-cell hematologic malignancy is not limited to CLL/SLL. Am J Clin Pathol 122:582-7 (2004).
7. Protection of Laboratory Workers from occupationally acquired infections. Second edition; approved guideline (2001). Villanova PA: National Committee for Clinical Laboratory Standards; Document M29-A2.
8. Procedures for the collection of diagnostic blood specimens by venipuncture- approved standard; Fifth edition (2003). Wayne PA: National Committee for Clinical Laboratory Standards; Document H3-A5.
9. Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; approved guideline (1998). Wayne PA: National Committee for Clinical Laboratory Standards; Document H42-A.

WARRANTY

This product is warranted only to conform to quantity and label specifications. There are no warranties that extend beyond the description on product label. Cytognos' sole liability is limited to either product replacement or refund of the purchase price.

EXPLANATION OF SYMBOLS

	Use by (use by YYYY-MM)
	Storage temperature limitation
	Consult instruction for use
RUO	For research use only
	Batch code
	Code number
	Manufacturer

PRODUCED BY

CYTOGNOS SL
Polígono La Serna, Nave 9
37900 Santa Marta de Tormes
Salamanca (Spain)
Phone: + 34-923-125067
Fax: + 34-923-125128
Ordering information: admin@cytognos.com
Technical information: support@cytognos.com

www.cytognos.com