CD117-FITC

Ref: CYT-117F4

For Research Use Only

INTENDED USE

CD117-FITC is a monoclonal antibody (MAb) labelled with fluorescein isothiocyanate (FITC) designed for flow cytometry use as a direct immunofluorescence reagent in the identification and enumeration of CD117 antigen-expressing cells. CYT-117F4 is a specific marker to detect leukemic cells committed to the myeloid lineage and therefore it represents a useful reagent in the characterization of acute leukaemias [(1)]. C-kit expression would be particularly relevant for the diagnosis of biphenotypic acute leukaemias as well since in these cases its association with myeloid lineage is greater than that of the CD13 and CD33 antigens.

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.

CD117 reacts specifically with human c-kit gene product (SCF receptor). The c-kit proto-oncogen (CD117) has been shown to be present in several cell types including normal and neoplastic haemopoietic cells [(2)]. The majority of CD117+ bone marrow (BM) cells (50- 70%), which mainly correspond to myeloid precursors, coexpress the progenitor-associated CD34 antigen. The c-kit+/CD34- cells mainly consist of immature cells, mast cells and CD34- erythroid precursors.

In acute leukaemias CD117 expression was initially associated with AML. Nevertheless, at present it is well established that CD117 expression may also be found in a relatively important proportion of T-ALL while it is usually absent in B-lineage ALL. The analysis of CD117 could be relevant for the investigation of minimal residual disease (MRD) since in combination with other antigens may be useful for the identification of leukaemia associated phenotypes in patients who achieved morphological complete remission. The combination of CD117 and other myeloid-associated antigens such as CD11b and CD15 may be of great help to monitoring MRD in AML patients although it defines a subpopulation of myeloid cells which are either absent or present at very low frequencies in normal human BM [(3)]. Additionally, some acute leukaemias express CD117 over-expression which is not detected in normal cells [(2)].

Moreover, different studies have shown that in around one-third of both multiple myeloma cases and patients with monoclonal gammopathy of undetermined significance myelomatous plasma cells display reactivity for CD117 [(4)].

BM mast cells are clearly identifiable on the basis of their light scatter properties and strong CD117 expression. BM mast cells studies result interesting for the diagnosis of adult indolent systemic mast cell disease [(5)] and mast cell leukaemia [(6)].

PRINCIPLES OF THE PROCEDURE

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.

Detection of targeted populations can be hindered by erythrocytes presence. This problem can be avoided by their removal using a red blood cell lysing solution previous to sample acquisition. Quicklysis™ (CYT-QL-1) erythrocyte lysing solution without fixatives is recommended since it requires no washing steps. Quicklysis™ use minimises sample handling and avoids centrifuge processed which are usually associated to cell losses [(7, 8)].

The CD117+ cell count is expressed as a percentage of total amount of lymphocytes or leucocytes 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 CD117 antibody conjugated with fluorescein isothiocyanate (FITC) is supplied in phosphate buffered saline with 0,1% sodium azide.

Clone: 104D2

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 [9]. 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

CD117-FITC 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.
- Quicklysis™ lysing solution.
- Wash buffer (phosphate buffered saline (PBS) containing 0,1% sodium azide).

Preparation

Whole blood sample must be taken aseptically by venipuncture [10, 11] in a sterilized tube containing an appropriate anticoagulant (use of EDTA is recommended). Flow cytometry analysis require one hundred (100) µL whole blood sample per tube, assuming a normal range of approximately 4 to 10 x 10^3 leucocytes per µL. Samples with high white blood cell count should be diluted with PBS to obtain an approximate cell concentration of 1 x 10^4 cells/µL. Store blood samples at 18-22ºC until they are to be tested. It is advisable to test blood samples within 24 hours after their extraction. Samples with suspended cell aggregates or haemolysed should be rejected.

1. Mix 100µL of peripheral blood with 10µL of CD117-FITC. In case of working with other body fluids with fewer cells, such as cerebrospinal fluid, bronchoalveolar lavage, gastric lavage, etc., start with an initial volume of 200 µL.
   To evaluate non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
2. Incubate in the dark at room temperature for 10 minutes.
3. Add 2 mL of Quicklysis™* erythrocyte lysing solution and incubate sample in the dark at room temperature for 10 minutes.
4. Acquisition in the flow cytometer should be performed within the first four hours after sample preparation. If samples are not acquired immediately after preparation, they should be stored in the dark at 2-8ºC. Instrument calibration must be done according to manufacturers' advice. Before acquiring samples, adjust threshold or discriminator to minimise debris and ensure that populations of interest are included. Also, previous to acquisition samples should be mixed on a vortex (low speed) to reduce cell aggregation.

*Note: The use of other lysing solutions may require elimination of lysed red blood cells. Follow lysing solution manufacturer recommended protocol.

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 lymphocyte or leucocyte 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 CD117+ 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.

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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
LOT	Batch code
REF	Code number
	Manufacturer

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