CD38 multi-epitope-FITC

Ref: CYT-38F2

CE

IVD

For in vitro diagnostic use

REAGENT COMPOSITION

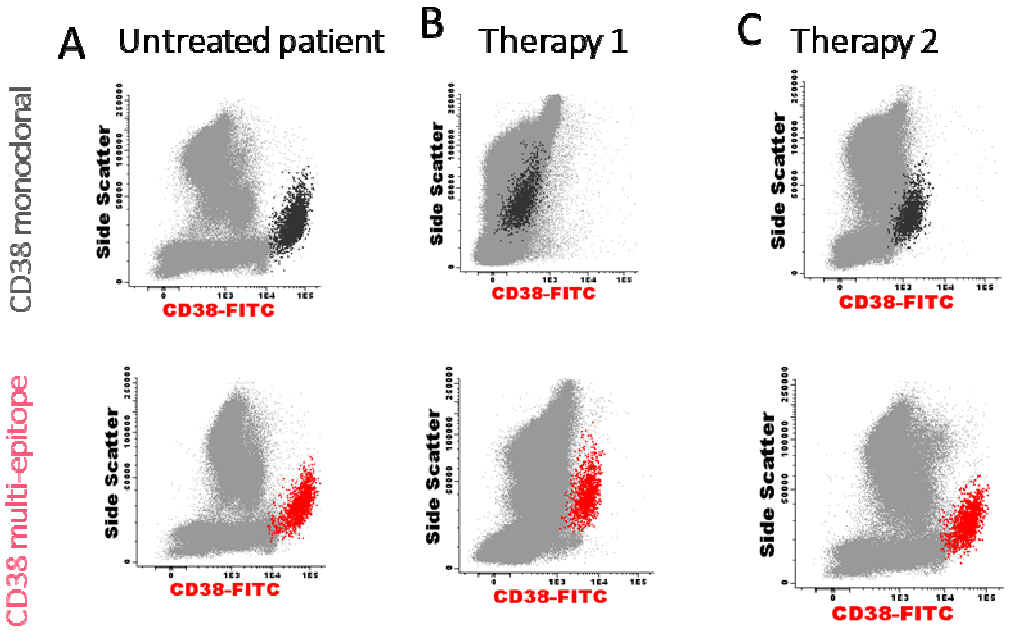
Purified pool of multi-epitope anti-CD38 antibodies labelled with fluorescein isothiocyanate (FITC) and supplied in phosphate-buffered saline (PBS) containing 1% (m/v) BSA and ≤0.09% (m/v) sodium azide.
Amount per vial: 0,25 ml (50 tests using 5 µl Ab per 10^6 cells)

INTENDED USE AND PERFORMANCE

CYT-38F2 is designed for Flow Cytometry (FC) use for the identification and enumeration of human CD38 antigen-expressing cells even when cells are hampered by other monoclonal antibody.
After an extensive selection of clones against different parts of the CD38 molecule, Cytognos has developed a multi-epitope reagent that allows the identification by FC of CD38 in plasma cells even if the patient is under treatment with Anti-CD38.

CD38, also known as ADP-ribosyl cyclase, is a type II transmembrane glycoprotein able to transform $NADP^+$ into cyclic ADP-ribose, $NAADP^+$ and ADP-ribose [1]. CD38 is expressed at variable levels on the majority of hematopoietic cells (during early differentiation and activation) and in some non-hematopoietic tissues [2]. It is expressed at high levels on plasma cells (CD38+++) and, due to its strong reactivity, CD38 is considered a "specific plasma cell marker" [3]; but it is also present on thymocytes, activated T cells, NKs monocytes, and basophils [4-6]. CD38 has been reported to play a role as regulator (positive and negative) of cell activation and proliferation, and to be involved in adhesion between lymphocytes and endothelial cells [2].
CD38 antibody is used in FC for diagnosis and monitoring of plasma cell disorders (e.g. multiple myeloma, MGUS, plasma cell leukemia, etc.). The simultaneous assessment of the expression of CD38 and CD138 represents the best combination of markers for the identification of plasma cells [7].
Anti-CD38 monoclonal antibodies continue to demonstrate promising results as new treatments for patients with multiple myeloma. Therapeutic anti-CD38 targets this molecule, highly expressed on the surface of multiple myeloma cells, hampering its detection by FC.
Cytognos has developed an **anti-human CD38 multiepitope reagent** containing a mixture of antibodies that allows the identification by FC of CD38 molecule on plasma cells, even in patients under anti-CD38 therapy.

Following is shown a comparative evaluation by FC of CD38-FITC monoclonal (dark grey dots) and CD38 multi-epitope-FITC (CYT-38F2; red dots). A) Bone marrow samples from untreated patients. B) and C) patients treated with two different therapies against CD38.



This reagent must be used by FC qualified personal.

STORAGE CONDITIONS

The reagent is stable until the expiration date shown on the label, when stored at 2-8º C. The reagent should not be frozen or exposed to direct light during storage or during incubation with cells. Keep the reagent vial in a dry place. Once opened, the vial must be stored in a vertical position to avoid any possible spillage.

WARNINGS, PRECAUTIONS AND LIMITATIONS

1. Reagents are not considered sterile.
2. For *in vitro* diagnostic use.

3. Alteration in the appearance of the reagent, such as the precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.
4. It contains 0,09% (m/v) sodium azide (CAS-No. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination of reagent or incorrect results may occur.
   Indication(s) of danger:

H302	Harmful if swallowed

   Safety advice:

P264	Wash thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P301+P312	If swallowed, call a poison center or doctor/physician if you feel unwell.
P301+P330	If swallowed, rinse mouth.
P501	Dispose of contents/container in accordance with local/regional/national/international regulation.

5. All patient specimens and materials with which they come into contact are considered biohazards and should be handled as if capable of transmitting infection [8], and disposed according to the legal precautions established for this type of product. Also recommended is handling of the product with appropriate protective gloves and clothing, and its use by personnel sufficiently qualified for the procedures described. Avoid contact of samples with skin and mucous membranes. After contact with skin, wash immediately with plenty of water.
6. Use of the reagent with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.
7. Do not use antibody beyond the expiration date on the label.
8. Any serious incident relating to the product must be reported to Cytognos S.L. as well as the competent professional authority of the Member State in which the user is established.

PREPARATION

This product is supplied ready to use.

PROCEDURE

Sample must be taken aseptically [9, 10] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). Add the appropriate volume of sample (blood or bone marrow aspirate) in order to stain $1x10^6$ cells in 50 µl. The analysis requires 50 µl of sample per tube, assuming a normal range of approximately 4 to 10 x 10^3 leucocytes per µl. For samples with a high white cell count, dilute samples with PBS to obtain a concentration of cells approximately equal to 1 x 10^4 cells/µl . Store the samples at 18-22ºC until they are to be tested. It is advisable to test samples within the 24 hours after their extraction. Hemolyzed samples or samples with suspended cell aggregates should be rejected.

Example of procedure (the user can titrate the reagent to suit the application):

1. Spin down the vial before each use.
2. Mix 50 µl of sample with 5 µl of CD38-FITC.
3. Incubate for 15 minutes at room temperature in the dark.
4. Add erythrocyte lysing solution and incubate the sample for 10 minutes at room temperature in the dark.
5. Acquire directly on the flow cytometer within the first hour of finishing the sample preparation. If the samples are not acquired immediately after preparation, they should be stored at 2-8ºC in the dark. Calibration of the instrument must be done according to the manufacturer's advice. Before acquiring samples, adjust the threshold or discriminator to minimize debris and ensure populations of interest are included. Before acquiring the sample on the flow cytometer, mix the cells on the vortex at low speed to reduce aggregation.

Flow cytometry analysis

Check that the cytometer is correctly aligned and standardized for light dispersion and fluorescent intensity, and that the right color compensation has been set following the instructions of the cytometer manufacturer. Cytognos recommends the use of the **analysis software Infinicyt™** [11], which is capable to use pattern recognition and store analysis strategies to apply in batch to other samples. You will find complete information about Infinicyt™ on the web site: www.infinicyt.com.

LIMITATIONS

- Blood samples should be stored at 18-22ºC and be tested within the 24 hours after they are obtained.
- It is advisable to acquire stained samples to the cytometer as soon as possible to optimize the results. Nonviable cells may stain nonspecifically. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction and loss of cells from the target population.
- When using whole blood procedures, all red blood cells may not lyse under following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red blood cells being counted as leukocytes.
- Results obtained by flow cytometry may be erroneous if the cytometer laser is misaligned or the gates are improperly set.
- Each laboratory should establish a normal range for leucocytes using its own test conditions.
- Certain patients may present special problems due to altered or very low number of certain cellular population.
- Cells separated from whole blood by means of density gradients may not have the same relative concentrations of cells as unseparated blood. This may be relatively insignificant for samples from individuals with normal white blood cell counts. In leukopenic patients, the selective loss of specific subsets may affect the accuracy of the determination.
- It is important to understand the normal pattern of expression of this antigen and its relation to the expression of other relevant antigens to carry out an adequate analysis [2-6].
- Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual who may be in an abnormal state of health may show the same leukocyte percentages as a healthy person. For this reason, it is advisable to use the test results in combination with other clinical and diagnosis data.

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. To evaluate the non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
- This product has been manufactured in accordance with standards of production and quality system of the ISO 13485:2012 standard.

ANALYTICAL EFFICIENCY

Reproducibility and Repeatability:

The results for 10 different samples stained with 2 different lots of the same reagent were compared. Results were analyzed independently by 2 technicians. Each pair of data for the same sample was analyzed, obtaining mean fluorescence intensity (MFI) and individual standard deviation (SD). SDs was combined to derive a grouped SD, grouped CV % and relative difference percentage that provides an estimate of repeatability and reproducibility. The results of the analysis are shown in the following charts:

POOLED SD (between lots)	POOLED CV % (between lots)	RELATIVE DIFFERENCE % (between lots)
2.913,72	26,38	-16,77

POOLED SD (between technicians)	POOLED CV % (between technicians)	RELATIVE DIFFERENCE % (between technicians)
2.057,66	18,30	19,98

Specificity and Sensitivity

To evaluate the cross-reactivity of the reagent with other cell populations, a study was performed on 10 blood samples from healthy donors, stained with CD38-FITC. The percentage of lymphocytes, monocytes and granulocytes stained with the mentioned mAb was evaluated. The results obtained are shown in the following table:

	CD38+ POPULATION	REFERENCE POPULATION
%Positive Population	0,39%	Plasma Cell
MFI	10119,48	

QC VERIFICATION

QC verification for each reagent lot has been performed the following methodology:

SAMPLE	PROTOCOL	POSITIVE POPULATION	ACQUISITION CYTOMETER
Peripheral Blood from healthy donors	EuroFlow settings and Bulk Lysis SOP	Plasma cells CD45+ / CD19 + / CD27+ / CD38+	FACS Canto II (Becton Dickinson)

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WARRANTY

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

EXPLANATION OF SYMBOLS

	Use by (YYYY-MM)
	Storage temperature limitation
	Keep out of sunlight
	Consult instructions for use
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Manufacter

PRODUCED BY

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