CD3

For In Vitro Diagnostic use

Fluorochrome	Reference	Clone	Isotype	Format
FITC	CYT-3F9	UCHT-1	Mouse / IgG1	1 ml/200 test
PE	CYT-3PE1	UCHT-1	Mouse / IgG1	1 ml/200 test
APC	CYT-3AP10	UCHT-1	Mouse / IgG1	1 ml/200 test
APC-C750	CYT-3AC750	UCHT-1	Mouse / IgG1	150 µl/50 test
PE-Cyanine5	CYT-3C4	UCHT-1	Mouse / IgG1	1 ml/200 test
PerCP-Cyanine5.5	CYT-3C9	UCHT-1	Mouse / IgG1	150 µl/50 test

INTENDED USE

CD3 reagent is a monoclonal antibody (mAb) conjugated with different fluorochromes (see table above) and designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express the CD3 antigen by flow cytometry. This reagent must be used by flow cytometry qualified personal.

PRINCIPLES OF THE PROCEDURE

Flow cytometry is an innovative technology by means of which different cell characteristics are simultaneously analyzed on a single cell basis. This is achieved by means of hydrodynamic focusing of cells that pass aligned one by one in front of a set of light detectors; at the same time they are illuminated by a laser beam. The interaction of the cells with the laser beam generates signals of two different kinds: those generated by dispersed light (FSC/SSC), which mainly reflects the size of the cell and its internal complexity, and those related to the emission of light by the fluorochromes. These signals become electric impulses which are amplified and registered as digital signals to be processed by a computer.

When the reagent is added to the sample, the fluorochrome-labelled mAb presents in the reagent bind specifically to the antigens they are directed against, allowing the detection by flow cytometry of the cell populations carried by the antigen.

The erythrocyte population, which could hinder the detection of the target population, is eliminated by the use of a red blood cell lysing solution previous to acquire the sample on the cytometer. The use of Quicklysis™ (CYT-QL-1) erythrocyte lysing solution is recommended, since it requires no further washing step and contains no fixative, therefore minimizing the handling of the sample and avoiding the cell loss associated to the centrifuge process.[1, 2].

The anti-CD recognizes the CD3 antigen present in T cells, and can therefore be used in the characterization studies for immunophenotyping of lymphocytes. These studies are widely applied for monitoring of the immunologic status of post-transplant patients and in the characterization and follow-up of immunodeficiencies, autoimmune diseases, leukemia etc [3, 4].

The T lymphocyte (CD3+) count is generally expressed as a percentage of the total amount of lymphocytes or leucocytes present in the sample which can itself be determined by flow cytometry based on its typical pattern of FSC/SSC (size/granularity or complexity). Because each flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION

Purified monoclonal CD3 antibody conjugated with different fluorochromes (see table above) is supplied in phosphate-buffered saline (PBS) containing 1% (m/v) BSA and ≤0.09% (m/v) sodium azide.

Clone: UCHT-1

Isotypes: Mouse / IgG1

Purification: Affinity chromatography

Size: 200 or 50 tests (1 ml or 150 µl per vial)

Usage: 5 µl (CYT-3F9, CYT-3PE1, CYT-3AP10 and CYT-3C4) or 3 µl (CYT-3AC750 and CYT-3C9) mAb per determination

Reagents are not considered sterile.

Last Revision: 8/25/2017

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 sample. Keep the reagent vial in a dry place. Once opened, the vial must be stored in a vertical position to avoid any possible spillage.

STABILITY

The expiration date of the reagent was determined by stability assays during use. The proper functioning of the reagent is guaranteed until the expiration date shown on the label if the storage conditions described above are met.

WARNINGS AND RECOMMENDATIONS

1. For *in vitro* diagnostic use.
2. This product is supplied ready to use. If it is altered by dilution or addition of other components, it will be invalidated 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-Nr. 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 [3], 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. 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.

PROCEDURE

Material included

Anti-CD3 mAb sufficient for the number of determinations shown in the vial (5 µl (CYT-3F9, CYT-3PE1, CYT-3AP10 and CYT-3C4) or 3 µl (CYT-3AC750 and CYT-3C9) mAb per determination).

Material required but not included

- Flow cytometer equipped with proper laser and band pass filter and appropriate computer hardware and software.
- Test tubes suitable for obtaining samples in the flow cytometer used. Usually tubes with a rounded bottom for 6 ml, 12x 75 mm are used.
- Automatic pipette and tips
- Chronometer
- Vortex Mixer
- Quicklysis™ lysing solution
- Wash buffer (phosphate buffered saline (PBS) containing ≤0.09% (m/v) sodium azide.

Preparation

Sample must be taken in a sterilized tube containing an appropriate anticoagulant (use of EDTA is recommended) [6, 7]. Flow cytometry analysis require 100 µl 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 samples at 18-22ºC until they are to be tested. It is advisable to test samples within 24 hours after their extraction. Samples with suspended cell aggregates or haemolysed should be rejected.

Recommended procedure:

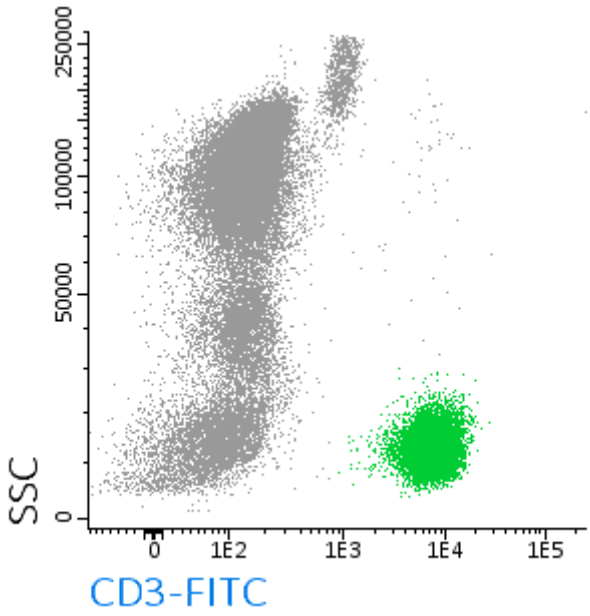
1. Spin down the vial before each use.
2. Mix 100µl of sample with 5 or 3 µl of anti-CD3 mAb (see table above).
3. Incubate for 15 minutes at room temperature in the dark.
4. Add 2 ml of Quicklysis™* erythrocyte lysing solution and incubate the sample for 10 minutes at room temperature in the dark.
5. Mix the cells by using the vortex at low speed to reduce aggregation. Acquire directly on the flow cytometer within the first four hours of finishing the sample preparation. If the samples are not acquired immediately after preparation, they should be stored at 2-8ºC in the dark.
6. Calibration of the instrument must be done according to the manufacturer's advice. Before acquiring samples on the flow cytometer, adjust the threshold or discriminator to minimize debris and ensure populations of interest are included.

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

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. Cytognos recommends the use of the **analysis software Infinicyt™** [8], 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.

Visually inspect the CD3 vs SSC dot plot: the T lymphocyte population (CD3+) should appear as a compact CD3+ cluster with low SSC. Do not proceed with analysis if the T-lymphocyte population is diffuse and if there is little or no separation between it and other clusters. The following figure shows representative flow cytometry data on peripheral blood (healthy individual) stained with the antibody. Stained cells (CD3+ shown on green color) and unstained populations (CD3- shown on grey color) are clearly identifiable.



The results are commonly reported as a percentage of the total of lymphocyte or leucocytes count present in the sample.

LIMITATIONS

- 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 samples to lytic reagents may cause white cell destruction and targeted population cell loss.
- When using whole blood lysing 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.
- 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 [9-11].
- 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.

EXPECTED VALUES

Each laboratory should establish its own normal reference ranges for T lymphocyte (CD3+) counts, since such values may be influenced by age, sex and race [12-14]. Based on the consulted bibliography and with a merely informative character, the percentage of CD3+ cells regarding the total of lymphocytes obtained by flow cytometry in healthy individuals is around 55-82% for children of 0-3 years of age [14] and 61-85% for adults between the ages of 18 and 70 [12].

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:

10 peripheral blood samples stained with 2 different lots of the same reagent were evaluated. Results were analyzed independently by 2 technicians. Each pair of data for the same sample was analyzed, obtaining the mean fluorescence intensity (MFI) and the standard deviation from which a grouped % CV and relative difference was calculated between lots and between technicians. The results of the analysis of CYT-3F9 are shown in the following charts:

SD (between lots)	GROUPED %CV (between lots)	% RELATIVE DIFFERENCE (between lots)
987,42	11,98	-16,95

SD (between technicians)	GROUPED %CV (between technicians)	% RELATIVE DIFFERENCE (between technicians)
2,52	0,03	0,01

Specificity/Sensitivity

The CD3 antigen is found on the cell surface of mature thymocytes and T lymphocytes in peripheral blood. 10 blood samples were stained with the antibody of interest and the percentage of positive cells for this marker [15] was evaluated and compared with a control reagent (Refrence). The results of the analysis of CYT-3F9 are shown in the following table:

	T LYMPHOCYTES	REFERENCE
%Positive Population	66,99	65,90
Range	(53,82 - 77,26)	(52,73 - 78,65)

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 SOPs	CD3+ T cells	FACS Canto II (Becton Dickinson)

QC verification applies in following reagents: CYT-3AP10 and CYT-3AC750.

REFERENCES

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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)
	Catalogue number
	Batch code
	Keep out of sunlight
	Storage temperature limitation
	Consult instructions for use
	Manufacter
	*In vitro* diagnostic medical device
	Contains sufficient for <n> tests
	Health hazard/Hazardous to the ozone layer

PRODUCED BY

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