QUICKLYSIS™

Ref: CYT-QL-1

CE

For In Vitro Diagnostic use

INTENDED USE

QUICKLYSIS™ is an erythrocyte lysing solution which provides complete and gentle lysis of erythrocytes after immunofluorescence staining of peripheral blood, bone marrow, cord blood or leukapheresis samples and prior to flow cytometric analysis.

SUMMARY AND EXPLANATION

Flow Cytometry is a powerful tool for the analytical and quantitative characterization of cells which provides rapid, quantitative and multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis. Flow cytometry is performed on cells in liquid suspension that have been incubated with fluorescently-labeled antibodies directed against specific cellular proteins. The relative fluorescence intensity of the positive cells indicates the amount of antibody bound to specific binding sites on the cells, and therefore provides a relative measure of antigen expression.

Efficient detection of leucocytes by flow cytometry depends on the elimination of interfering cells. In clinical laboratories, whole blood lysis methods have essentially replaced Ficoll density gradient separation because of shorter sample preparation time and less handling of whole blood.

PRINCIPLES OF THE PROCEDURE

Flow cytometry (FC) 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 present in the cell. 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 monoclonal antibodies presents in the reagent bind specifically to the antigens they are directed against, allowing the detection by FC 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™ 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 reagent contains no fixative.

REAGENT PROVIDED

CYT-QL-1 is provided as 500 ml of a proprietary buffered solution. This quantity is sufficient for 250 tests (2 ml QUICKLYSIS™ per test)
Reagent is not considered sterile.

STORAGE CONDITIONS

QUICKLYSIS™ is stable until the expiration date shown on the label when it is stored at room temperature (20-25ºC). The pH of the reagent may increase during storage, which may affect the position of cells in an FSC/SSC dot plot. The pH of the reagent should be between 7 and 7,4. If the pH is out this range, it should be adjusted by adding diluted solutions of HCl or NaOH.

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. The reagent is stable until the expiration date shown on the label if it is properly stored. Do no use after the expiration date shown on the label. If the reagents are stored in conditions different from those recommended, such conditions must be validated by the user.
4. 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.
5. It contains 0,1% 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.
    - 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).
    - Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in metal drains where explosive conditions may develop.
6. 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.
7. 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.

PROCEDURE

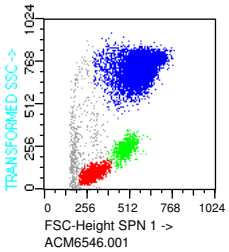
Material included
QUICKLYSIS™ is sufficient for 250 determinations (2 ml QUICKLYSIS™ to 10^6 cells).

Material required but not included
- 488 nm ion argon laser-equipped flow cytometer 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 (100µL) and tips.
- Micropipette with tips.
- Timer
- Vortex Mixer
- Fluorochrome-conjugated antibodies to leucocyte antigens
- Wash buffer as phosphate buffered saline (PBS) containing 0,1% sodium azide.

Preparation
Whole blood sample must be taken aseptically by means of a venipuncture[4, 5] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). The analysis requires one hundred (100) 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.
Bone marrow sample should be pass 3 or 4 times through a syringe in order to disaggregate cell clumps. Perform a white blood cell count of the sample and dilute samples with PBS to obtain a concentration of cells approximately equal to 1 x 10^4 cells/µ

1. For each sample, combine appropriated amounts of fluorochrome-conjugated antibodies and blood per tube as directed in the specific package insert.
2. Incubate for 10 minutes at room temperature in the dark.
3. Add 2 ml of Quicklysis™ erythrocyte lysing solution and incubate the sample for 10 minutes at room temperature in the dark.
4. 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 in the dark at 2-8ºC. 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 (FSC/SSC on linear scale) and fluorescent intensity (FL1, FL2, FL3 FL4 on logarithmic scale) and that the right color compensation has been set following the instructions of the cytometer manufacturer.
The following figure shows representative flow cytometry data on peripheral blood (healthy individual) treated with QUICKLYSIS™. This reagent sometimes separates neutrophils into two discrete populations with the same SSC characteristics but different FSC. If neutrophils FSC/SSC gating is applied during analysis of data, care must be taken to set the gate region to include both FSC populations.



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 on 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 leucocytes.
- 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 or lymphocytes using its own test conditions. The data for the reagent's performance have been obtained from whole blood samples collected with EDTA as anticoagulant. The reagent's performance may be affected by the use of other anticoagulants.
- Certain patients may present special problems due to altered or very low number of certain cellular population.
- Abnormal states of health are not always represented by abnormal percentages of certain leucocyte populations. An individual who may be in an abnormal state of health may show the same leucocyte 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 must establish its own normal reference ranges for leucocyte and lymphocyte subsets counting, since such values may be influenced by age, sex and race [6-7]. Peripheral blood samples from 80 normal adult between the ages of 18 and 60 years were collected to study reference ranges for different lymphocyte subpopulations. Samples were stained with LYMPHOGRAM® and red blood cells were lysed with QUICKLYSIS™. The reference ranges for different lymphocyte subsets shown in the following table are expressed

as the percentage of the lymphocyte subpopulations of the total lymphocyte count. These data were determined using the analysis software CYTORAMA- LYMPHOGRAM®.

LYMPHOCYTE SUBSET	MEAN	MAX VALUE	MIN VALUE
%Total T lymphocytes (CD3+)	76,6	88,68	50,87
% T lymphocytes CD4+CD8-	48,1	67,11	24,43
% T lymphocytes CD4-CD8++	23,7	53,64	12,16
% T lymphocytes CD4-CD8-+	4,0	7,48	1,42
% T lymphocytes CD4+CD8+	0,9	6,02	0,14
%Total B lymphocytes (CD19+)	13,0	25,5	4,98
% NK cells (CD56+CD3-)	9,7	24,4	2,07

QUALITY CONTROL

- To obtain optimum results it is advisable to verify the precision of pipettes and that the cytometer is correctly calibrated.
- The different fluorochromes, as for example fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), etc., emit in different wavelengths but show a certain spectral overlapping which must be corrected by means of electronic compensation if combinations of different antibodies are used conjugated with these fluorochromes. The optimum levels of compensation can be established by analysis in a dot-plot diagram of cells from healthy individuals stained with mutually exclusive antibodies conjugated with the fluorochromes to be used in the test.

PERFORMANCE CHARACTERISTICS

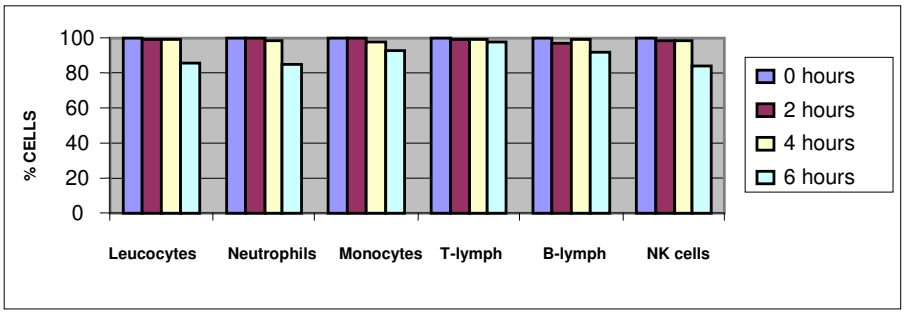
Reproducibility:

10 repeated measures from three peripheral blood samples representing high, medium and low lymphocyte counts were evaluated. Samples were stained with LYMPHOGRAM® (CYT-C-001) and red blood cells were lysed with QUICKLYSIS™. In the following table are shown the mean percentage of the different lymphocyte subsets of the total lymphocytes count, the standard deviation and the coefficient of variation obtained for each of the three levels studied:

Lymphocytes level	% Total T lymphocytes (CD3+)	% Total T lymphocytes CD4+CD8-	% Total T lymphocytes CD4-CD8+	% B lymphocytes (CD19+)	% NK cells (CD56+CD3-)
High	83,39 ± 0,49 (CV=0,59)	43,19 ± 0,73 (CV=1,69)	30,32 ± 1,05 (CV=3,46)	11,53 ± 0,43 (CV=3,73)	5,08 ± 0,24 (CV=4,72)
Medium	81,10 ± 0,42 (CV=0,52)	48,00 ± 0,52 (CV=1,08)	30,37 ± 0,88 (CV=2,90)	14,42 ± 0,31 (CV=2,15)	4,48 ± 0,42 (CV=9,38)
Low	86,53 ± 0,59 (CV=0,68)	58,72 ± 0,88 (CV=1,50)	24,91 ± 0,59 (CV=2,37)	1,72 ± 0,17 (CV=9,88)	11,72 ± 0,54 (CV=4,61)

Stability of samples processed with QUICKLYSIS™

To evaluate the stability of the samples processed with QUICKLYSIS™, 5 peripheral blood samples were tested. Samples were stained with the monoclonal antibody combination CD45-FITC/CD56-PE/CD19-PECy5/CD3-APC and red blood cells were lysed with QUICKLYSIS™. Once processed, the samples were stored at 2-8ºC until their acquisition on the flow cytometer. To check the stability of the processed sample, the percentage of leucocytes, neutrophils, monocytes, T lymphocytes, B lymphocytes and NK cells were evaluated for the same sample on acquiring it immediately, 2 hours, 4 hours and 6 hours after its preparation. The results showed that it is recommended to acquire the samples within the first four hours of finishing the sample preparation.



White cell recovery

Ten peripheral blood samples were treated with QUICKLYSIS™ and analyzed for white cell recovery using a haematology analyzer. In the following table is described the leucocyte counts prior to and following the lysing of red blood cells expressed as cells x 10^3 / µl, and the percentage of leucocytes recovered. White cell recovery average obtained with QUICKLYSIS™ was of 96,81%.

Sample	Leucocyte count prior to lysis	Leucocyte count following lysis	Percent recovered		Sample	Leucocyte count prior to lysis	Leucocyte count following lysis	Percent recovered
1	9,1	8,82	96,92%		6	11,6	11,55	99,57%
2	10	9,87	98,70%		7	12	11,13	92,75%
3	16,9	15,75	93,20%		8	6,9	6,9	100%
4	12,4	11,76	94,84%		9	8,8	8,4	95,45%
5	13	12,6	96,92%		10	9,9	9,87	99,70%

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.

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