

# OptiLyse<sup>®</sup> C Lysis Solution

REF A11895  
200 tests; 100 mL  
0.5 mL / test



## ENGLISH

	Specifications Ready for use OptiLyse C Lysis Solution
Formulation	Buffered liquid solution containing 1.5% formaldehyde
Volume	100 mL
Number of vials	1 vial
Volume per test	0.5 mL

## USE

This reagent is intended for the lysis of red blood cells in the preparation of biological samples for flow cytometry analysis after staining of leucocytes with fluorescent antibodies. Use of OptiLyse C is limited to samples for analysis using Beckman Coulter flow cytometers and is compatible with so-called «no wash» staining and lysis procedures so long as duly calibrated fluorescent antibodies are used for this purpose.

## PRINCIPLE

The biological sample containing red blood cells for lysis is incubated in the presence of the OptiLyse C solution, which results in the lysis of red blood cells accompanied by the fixation of leucocytes.

A specific staining of leucocytes is first achieved by incubating the sample with the antibody or antibodies selected. The red cells are then removed by lysis and the leucocytes, which are fixed during this stage, are analyzed by flow cytometry.

Preparations must be stored between 2 and 8°C and rapidly analyzed by flow cytometry without prior washing being necessary.

The flow cytometer analyzes light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow-angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

## STORAGE AND STABILITY

OptiLyse C is stored between 18 and 25°C.

Vial closed, the reagent is stable up to the expiry date shown on vial.

After opening, the reagent is stable for 90 days.

## PRECAUTIONS

1. Do not use the reagent beyond expiry date.
2. Do not store in the refrigerator, do not freeze.
3. Minimize exposure to light during incubation with fluorescent antibodies.
4. Avoid microbial contamination of the reagents, or false results may occur.

5. Formaldehyde is toxic and allergenic. It is thought to be a carcinogenic agent. Never pipette by mouth and avoid all contact with the skin, mucosae, eyes and clothing
6. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
7. Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

## SAMPLES

Venous blood or bone marrow samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant or ACD or heparin.

The samples should be kept at room temperature (18 – 25°C) and not shaken. The sample should be homogenized by gentle agitation prior to taking the test sample.

The samples must be analyzed within 24 hours of venipuncture.

## METHODOLOGY

### NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material required for sampling. Automatic pipettes with disposable tips for sampling of 20, 100, 500 and 1000 µL volumes.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- Specific fluorescent antibodies.
- Isotypic controls.
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Fixation reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

### PREPARATION OF SAMPLES

The leucocyte content in the sample must be less than 10<sup>4</sup> cells / µL (10<sup>10</sup> cells / L). If necessary, dilute in PBS to bring the leucocyte concentration to 5 x 10<sup>3</sup> cells / µL (5 x 10<sup>9</sup> cells / L).

The red cell concentration in the sample must be less than 6 x 10<sup>6</sup> cells / µL (6 x 10<sup>12</sup> cells / L). If necessary, it is recommended that the sample be diluted in PBS to bring the red cell concentration down to 5 x 10<sup>6</sup> cells / µL.

If the leucocytic and erythrocytic concentrations need adjusting, the highest dilution must be carried out. The conjugated antibody staining procedures use 100 µL of sample (prediluted or otherwise) per tube.

## PROCEDURE

### Preparation of the reagent

No preparation is necessary. Use OptiLyse C directly from the vial.

### Procedure

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed with the isotypic control corresponding to the specific stain selected.

**NB:** The so-called «no wash» staining and lysis procedure described in stages 1 to 9 below is useful when duly calibrated fluorescent antibodies are used for this purpose.

1. Into each test tube, add the amount of antibody recommended by the manufacturer to stain 5 x 10<sup>5</sup> leucocytes.
2. Into each control tube, add the amount of isotypic control recommended by the manufacturer to stain 5 x 10<sup>5</sup> leucocytes.
3. Add 100 µL of the test sample (prediluted or otherwise) to both tubes. Vortex the tubes gently.
4. Incubate under the conditions set out in the data sheet of the antibodies used.
5. Add 0.5 mL of OptiLyse C and vortex immediately for 1 second.
6. Incubate for 10 minutes at room temperature (18 – 25°C) protected from light.
7. Add 0.5 mL of PBS and vortex.
8. Leave to incubate for at least 5 minutes at room temperature and away from light.
9. Analyze samples in the flow cytometer.

**NB:** If the preparations need to be washed, do not analyze before performing steps 10 and 11.

10. Centrifuge for 5 minutes at 300 x g at room temperature.
11. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 or 1 mL of PBS made up with 0.1 % formaldehyde. A 0.1 % formaldehyde PBS solution can be prepared by diluting 12.5 µL of the IOTest 3 Fixative Solution (Ref. A07800) at its ten-fold concentration in 1 mL of PBS.

**NB:** In all cases, keep the preparations between 2 and 8°C and away from light until the flow cytometric analysis, which should take place within 24 hours.

## PERFORMANCE

### Lymphocyte Purity and Recovery

Lymphocyte purity and recovery have been evaluated according to the recommendations of the CDC (1). The blood of 10 healthy donors, sampled in K<sub>3</sub>EDTA, was doubly stained by means of a mixture of CD45-FITC and CD14-PE monoclonal antibodies, specially calibrated for a procedure of so-called « no wash» staining and lysis. The means obtained with regard to recovery and purity of lymphocytes, as well as the ranges are given in the following table:

Recovery (%)	Purity (%)
96.8	94.8
96.2 / 97.3	94.3 / 95.1

### INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage and of the Mean Fluorescence Intensity (MFI) of CD45<sup>+</sup>CD14<sup>-</sup> lymphocytes were carried out on the same sample (total blood from a healthy donor). The so-called « no wash» staining and lysis procedure was used. The results obtained are summarized in the following table:

Whole blood	Number	Mean (%)	SD	CV (%)
Percentage CD45 <sup>+</sup> CD14 <sup>-</sup> Lymphocytes	12	99.9	0.04	0.0
MFI CD45 <sup>+</sup> CD14 <sup>-</sup> Lymphocytes	12	30.78	0.28	0.9

### INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample (total blood from a healthy donor), 12 measurements of the percentage and of the Mean Fluorescence Intensity (MFI) of CD45<sup>+</sup>CD14<sup>-</sup> lymphocytes were carried out by 2 technicians. The preparations were analyzed using 2 different cytometers. The so-called « no wash» staining and lysis procedure was used. The results obtained are summarized in the following tables:

Cytometer n° 1:

Whole blood	Number	Mean (%)	SD	CV (%)
Percentage CD45 <sup>+</sup> CD14 <sup>-</sup> Lymphocytes	12	99.9	0.04	0.0
MFI CD45 <sup>+</sup> CD14 <sup>-</sup> Lymphocytes	12	30.78	0.28	0.9

Cytometer n° 2:

Whole blood	Number	Mean (%)	SD	CV (%)
Percentage CD45 <sup>+</sup> CD14 <sup>-</sup> Lymphocytes	12	99.0	1.49	1.5
MFI CD45 <sup>+</sup> CD14 <sup>-</sup> Lymphocytes	12	37.65	0.20	0.5

### LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks have not been correctly compensated for and if the regions have not been carefully positioned.
2. Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert leaflet and compatible with good laboratory practices.
3. In the case of a hyperleucocytosis, dilute the specimen in PBS so as to obtain a value of  $5 \times 10^9$  leucocytes/L.
4. In the case of a polyglobulinaemia, dilute the specimen in PBS so as to obtain a value of  $5 \times 10^{12}$  red blood cells/L.
5. OptiLyse C solution must be brought to room temperature (18 – 25°C) before use.
6. Verify the preparations using the naked eye to assess the efficacy of lysis. If they are cloudy or if the light diffraction histograms are unusual, it may be that lysis is incomplete.
7. The erythroblasts may be incompletely lysed and appear on a light diffraction histogram in the same location as the leucocytes.
8. In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is advisable to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

### MISCELLANEOUS

See the Appendix for examples and references.

### TRADEMARKS

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### MANUFACTURED BY:

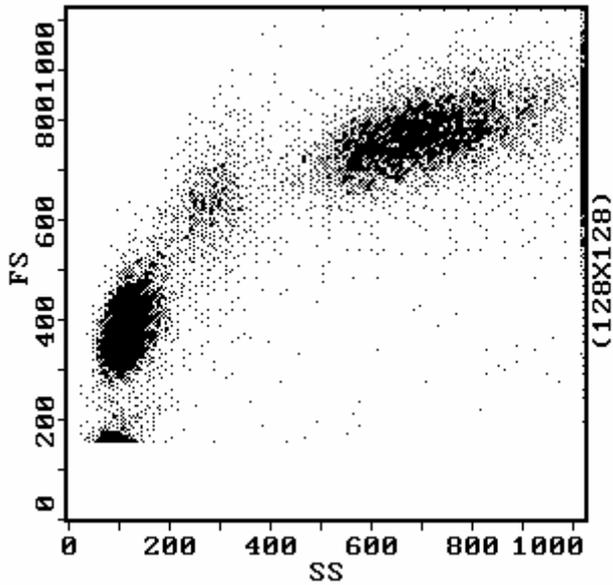
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**EXAMPLES**

The graph below is a biparametric representation (Forward Scatter vs. Side Scatter) of a normal whole blood sample lysed with OptiLyse C (Ref. A11895) using a no wash procedure.



Acquisition and analysis are with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software.

**REFERENCES**

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