For Research Use Only. Not for use in diagnostic procedures.
Product is manufactured in accordance with current Good Manufacturing Practices.

REF AE238377 Technical Data Sheet EN

Identification of the reagent

Name	CyLyse™ FXP
Ref. No.	AE238377
Content	25 mL Fixation Buffer (Ref. No. BW901813) 25 mL Permeabilization Buffer (Ref. No. BW412407)

Product Description

CyLyse™ FXP is a set of two ready-to-use reagents, Fixation and Permeabilization Buffer. This includes fixing and permeabilising of the cytoplasmic membrane of leukocytes and is used for red blood cell lysis in the preparation of biological samples from human peripheral blood after staining leukocytes with fluorochrome-conjugated antibodies prior to the flow cytometry analysis.

Principle of the procedure

Human leukocytes are stained with fluorochrome-conjugated antibody reagents that specifically bind to the antigenic determinants on the cell surface. The surface stained leukocytes are fixed with Fixation Buffer. Erythrocytes are lysed with deionized water and the remaining leukocytes are pelleted by centrifugation. The sediment is resuspended in Permeabilization Buffer and mixed with fluorochrome-conjugated antibody reagents against intracellular antigens. Antibodies enter the intracellular compartment and bind to their specific targets. The unbound antibodies are removed by washing and the cells are analyzed by a suitably equipped flow cytometer.

Storage and shelf life

Unopened product

Store CyLyse™ FXP at 2-25 °C. Do not freeze. Avoid prolonged exposure to light. Do not use after the expiration date stated on the label.

After first opening

CyLyse™ FXP retains its performance characteristics after having been placed into use for a minimum of 12 months.

Components

Fixation Buffer is provided in one vial containing 25 mL of a proprietary buffered fixative containing ≤ 5 % (v/v) formaldehyde.

Permeabilization Buffer is provided in one vial containing 25 mL of a proprietary buffered permeabilization solution and detergents.

The reagents are sufficient for 100 staining reactions.

Evidence of deterioration

Avoid contamination of reagents. In case of components deterioration seen as a visible precipitation or discoloration of the reagent or if data obtained show any performance alteration, please contact the Technical Support of your local Sysmex representative.

Any problem that has occurred in relation to the product shall be reported by the user to the manufacturer.

Precaution and warnings

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet (available at http://www.sysmex-partec.com/services).

Always meet the national and international guidelines and regulatory standards for PPE (personal protective equipment).

Warning symbols


GHS07


GHS08

Signal word

DANGER

Warnings

H302	Harmful if swallowed.
H317	May cause an allergic skin reaction.
H341	Suspected of causing genetic defects.
H350	May cause cancer.

Precautions

P201	Obtain special instructions before use.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.

Additional required equipment

Instrument	Flow cytometer equipped with appropriate computer hardware and software. The flow cytometer must be equipped to detect forward scatter (FSC) and side scatter (SSC).
Required laboratory equipment	Vortex mixer, Centrifuge, Material necessary for the collection of whole blood, Disposable test tubes (e.g. 12 × 75 mm) for staining of samples, Pipettes with disposable tips for 10, 100 and 1000 µL, Adequate personal protective equipment
Required reagents	Fluorochrome-conjugated antibody reagents (e.g. Sysmex CyFlow™ antibody reagents), Phosphate buffered saline (PBS; pH 7.4), Deionized water

Other materials can be required. Refer to the appropriate antibody reagent Instructions for Use (IFU) for more information.

Reagent preparation

CyLyse™ FXP is ready to use. If the CyLyse™ FXP is stored at 2-8 °C allow the reagent to warm up to room temperature before use.

Primary sample collection, handling and storage

⚠ ***WARNING*** *Consider all biological specimens and materials which come in contact with them as biohazardous. Specimens should be handled as potentially infectious and disposed in accordance with federal, state and local regulations.*

Collect whole blood in a sterile tube with K3 or K2 EDTA as anticoagulant. Follow the antibody reagent IFU for sample handling and storage.

Disposal

All disposables, which have been in contact with biohazardous material, must be decontaminated and disposed of according to local legislations and laws. Clean and disinfect contaminated surfaces immediately, use appropriate procedures of decontamination. Always dispose blood samples, assays and accessory fluids after expiration of the maximal storage time.

Examination procedure

1. Stain 100 µL of whole blood following instructions in the antibody reagent IFU (e.g. Sysmex Partec CyFlow™ antibody reagents).

2. Add 250 µL of Fixation Buffer to the tube and vortex gently.
3. Incubate for 10 minutes at room temperature (18-28 °C) in the dark.
4. For red blood cell lysis, add 3 mL of deionized water (18-28 °C) to the tube and vortex gently.
5. Incubate for 10 minutes at room temperature (18-28 °C) in the dark.
6. Centrifuge tubes for 5 minutes at 300 g and remove the supernatant by decanting.
7. Add 250 µL of Permeabilization Buffer.
8. Add antibody reagents intended for intracellular staining (e.g. Sysmex Partec CyFlow™ antibody reagents) and vortex gently.
9. Incubate for 15 minutes at room temperature (18-28 °C) in the dark.
10. Add 2 mL of PBS to the tube and vortex gently.
11. Centrifuge tubes for 5 minutes at 300 g and remove the supernatant by decanting.
12. For subsequent analysis, resuspend the cell pellet in a sufficient volume of PBS appropriate for your flow cytometer.
13. For later analysis, follow instructions in the antibody reagent IFU.

Limitations

The test is intended for professional and appropriately trained users in laboratories performing flow cytometry analysis.

In case of hyperleukocytosis, it is recommended to dilute blood samples with PBS to a concentration of 5 x 10^6 leukocytes/mL [1, 2, 3].

In certain disease states, such as haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is recommended to isolate mononucleated cells using a density gradient (e.g. Ficoll) prior to staining [4, 5, 6, 7, 8, 9].

Samples with nucleated red blood cells may show incomplete lysis of red blood cells. This may also occur when assaying blood samples from patients with certain hematologic disorders in which red cells are difficult to lyse, as in myelofibrosis, sickle-cell anemia or thalassemia [4, 5, 7, 10, 11].

The flow cytometer may produce false results, if the device has not been aligned and maintained appropriately.

Data may be incorrectly interpreted, if fluorescent signals were compensated wrongly or if gates were positioned inaccurately.

Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the Technical Data Sheet and compatible with good laboratory practices.

Literature references

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4. T. Constantino. Nucleated RBCs-Significance in the Peripheral Blood Film, Laboratory Medicine, Volume 31, Issue 4, 223–229 (2000)
5. S. Buoro. Evaluation of nucleated red blood cell count by Sysmex XE-2100 in patients with thalassaemia or sickle cell anaemia and in neonates, Blood Transfus., 13(4): 588-94 (2015)
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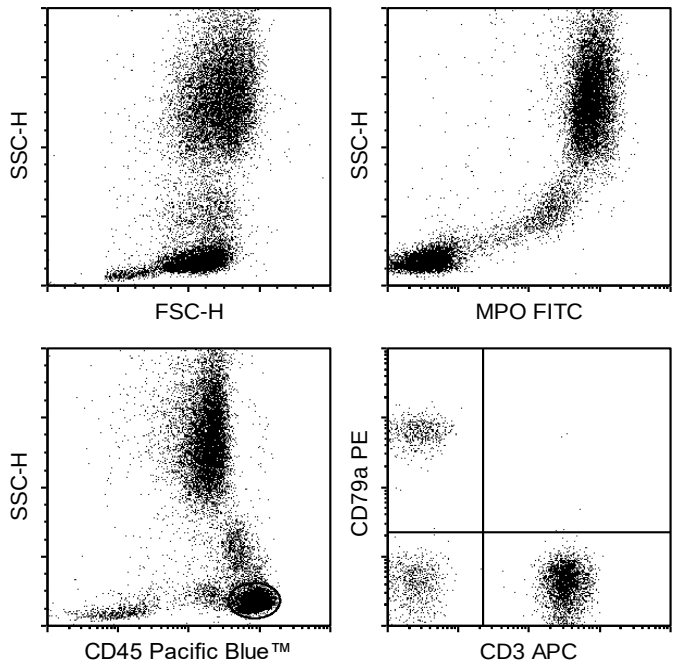
Representative data

The following representative data was obtained using human peripheral whole blood stained with Sysmex Partec CyFlow™ antibody reagents (CD3 APC, CD79a PE, Myeloperoxidase (MPO) FITC and CD45 Pacific Blue™) and treated with CyLyse™ FXP. The data was collected on a Sysmex Partec flow cytometer equipped with violet (405 nm), blue (488 nm) and red (638 nm) lasers.



Symbols

REF	Reference number		Legal manufacturer		Caution
LOT	Batch code		Temperature limitation		Use by
	Keep away from sunlight				

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