Read and follow instructions carefully.
Note: Changes to previous version highlighted

1 Identification of the IVD reagent

Name	CyLyse™ FXP
Ref. No.	BM900123
UDI-DI	04250878904405
Content	25 mL Fixation Buffer (Ref. No. AR134949)
	25 mL Permeabilization Buffer (Ref. No. CY078768)

2 Intended purpose

IVD For In Vitro Diagnostic Use.

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. CyLyse™ FXP is also intended to be used for in vitro diagnostic purposes by healthcare professionals and properly trained personnel in a laboratory environment and can be used for manual sample preparation by a user or with a sample preparation system.

3 Use in combination with other products

CyLyse™ FXP is used in combination with Sysmex CyFlow™ antibody reagents and enables their intended purpose.

4 Principle of the procedure

Human leukocytes are stained with Sysmex CyFlow™ 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 Sysmex CyFlow™ 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 analysed by a suitably equipped flow cytometer.

5 Storage and shelf life

5.1 Unopened product

Store CyLyse™ FXP at 2-28 °C in the dark. Do not freeze or expose to light. Do not use after the expiration date stated on the label.

5.2 After first opening

The shelf life after first opening is the same as the shelf life for unopened reagent if stored at stated storage conditions and used according to the instructions in this document.

6 Components

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

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

The reagents are sufficient for 100 staining reactions.

7 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. In case of serious incidents, please contact the manufacturer and a competent authority.

8 Precautions 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 personal protective equipment.

8.1 Warning symbols

GHS07

GHS08

8.2 Signal Word

DANGER

8.3 Warnings

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

8.4 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.

9 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).
	Optional: Sample preparation system (e.g. Sysmex Sample Preparation System PS-10)
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
Reagents:	Sysmex CyFlow™ antibody reagents
	Phosphate-buffered saline (PBS; pH 7.4)
	Deionized water

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

10 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.

11 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.

12 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.

13 Examination procedure

13.1 Manual sample preparation procedure:

1. Stain 100 µL of whole blood following instructions in the Sysmex CyFlow™ antibody reagents IFU.
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 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.

13.2 Automated sample preparation procedure:

CyLyse™ FXP is suitable to be used together with Sysmex Sample Preparation System PS-10. Refer to the instrument IFU for more information.

14 Limitations

Certain drugs in the patient's blood (given as medication or drugs of abuse) might interfere with the measurement procedure [1].

In case of hyperleukocytosis, it is recommended to dilute blood samples with PBS to a concentration of 5 x 10^6 leukocytes/mL [2-4].

Common sample abnormalities such as hyperbilirubinemia and lipemia might interfere with specific flow cytometry applications [1,5].

In certain disease states, such as hemoglobinopathies, lysis of red blood 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 [6-11].

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 blood cells are difficult to lyse, as in myelofibrosis, sickle-cell anemia or thalassemia [6,7,9,12,13].

Antibody staining prior to red blood cell lysis might be impaired by in vivo hemolysis caused by certain disorders (paroxysmal nocturnal hemoglobinuria, spherocytosis, autoimmune hemolytic anemia) [1,14-22].

Presence of proteins (e.g. albumin) or endogenous antibodies (e.g. human anti-animal antibodies) may interfere with the performance of the immunoassay [1,23-32].

The centrifugation and decantation steps may lead to a non-specific loss of cells and may not be suitable for absolute cell count determination.

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 IFU and compatible with good laboratory practices. This includes the avoidance of contaminations from various sources such as sample collection and preparation material.

15 Literature references

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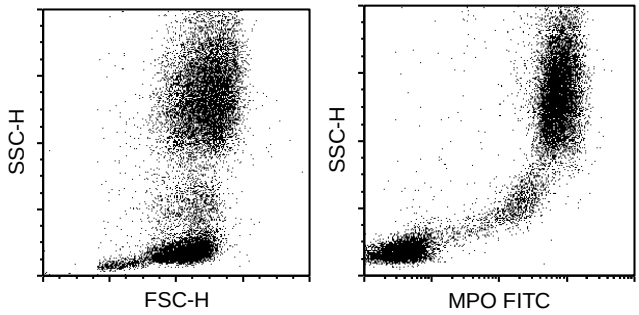
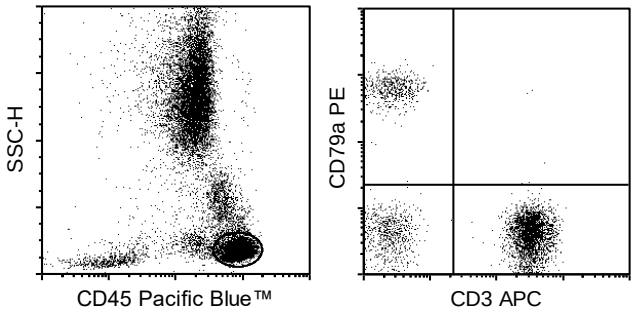
16 Representative data

The following representative data was obtained using human peripheral whole blood stained with Sysmex 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 flow cytometer equipped with violet (405 nm), blue (488 nm), and red (638 nm) lasers.





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18 Symbols

REF	Reference number		Manufacturer	Danger	Signal word: Danger
LOT	Batch code	IVD	In vitro diagnostic medical device	CONTENT	Content of kit
	Use-by date		Temperature limit	Componentes: 1. Fixation Buffer: Formaldehído < 5 %; Metanol < 2,5 % Composição: Formaldehyd < 5 %; Metanol < 2,5 % 2. Permeabilization Buffer: Caseína sódica ≤ 5 %; Cloruro de sodio 0,8 % Caseína sódica ≤ 5 %; Cloreto de sódio 0,8 %	Component information for various Latin-American countries
	Consult instructions for use		Keep away from sunlight	Advertencias y precauciones: Antes de usar, lea atentamente las Instrucciones del Interior y la Ficha de Datos de Seguridad. Advertências e precauções: Antes de usar, ler atentamente a Bula e a Ficha de Informações de Segurança de Produtos Químicos. A Ficha de Informações de Segurança de Produtos Químicos deste produto químico perigoso pode ser obtida por meio de acesso ao site www.sysmex.com.br. Centro de Informacoes Toxicológicas de Curitiba: 0800410148	Safety information for various Latin-American countries
CE	CE mark	UKCA	UKCA mark	**PELIGRO** H302: Nocivo en caso de ingestión. H317: Puede provocar una reacción alérgica en la piel. H341: Se sospecha que provoca defectos genéticos. H350: Puede provocar cáncer. P201: Solicitar instrucciones especiales antes del uso. P280: Llevar guantes/prendas/gafas/máscara de protección. P308+P313: EN CASO DE exposición manifiesta o presunta: Consultar a un médico. **PERIGO** H302: Nocivo se ingerido. H317: Pode provocar reações alérgicas na pele. H341: Suspeito de provocar defeitos genéticos. H350: Pode provocar câncer. P201: Obtenha instruções específicas antes da utilização. P280: Use luvas de proteção/roupa de proteção/proteção ocular/proteção facial. P308+P313: EM CASO DE exposição ou suspeita de exposição: Consulte um médico.	Hazard and precautionary statements for various Latin-American countries
UDI	Unique device identifier	UK RP	UK Responsible Person	Información del product: Una solución de lisado para la fijación y permeabilización para citometría de flujo. Informações sobre o produto: Uma solução de lise para fixação e permeabilização para citometria de fluxo.	Product information for various Latin-American countries

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