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

1 Identification of the IVD reagent

Name	CyLyse™ FX
Ref. No.	BD303500
UDI-DI	04250878904382
Content	50 mL

2 Intended purpose

IVD For In Vitro Diagnostic Use.

CyLyse™ FX is intended to be used as a lysing solution with a fixative for lysing of red blood cells following antibody staining of human peripheral blood cells prior to flow cytometric analysis. CyLyse™ FX 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™ FX is used in combination with Sysmex CyFlow™ antibody reagents and enables their intended purpose.

4 Principle of the procedure

Leukocyte analysis and detection in human peripheral blood requires elimination of interfering cells, mainly erythrocytes. Direct blood sample staining followed by red blood cell lysis and leukocytes fixation is a fast and easy method for whole blood flow cytometry analysis.

5 Storage and shelf life

5.1 Unopened product

Store CyLyse™ FX (10x concentrate) 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 Product 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 for Use (IFU).

5.3 Diluted product

Diluted CyLyse™ FX is stable for one month when stored at 18-28 °C in the dark. Do not freeze or expose to light. Refer to section *10 Reagent preparation* for more information.

6 Components

CyLyse™ FX (10x concentrate) is provided as 50 mL of a proprietary buffered clear and colorless solution containing 20-30 % (v/v) diethylene glycol, < 15 % (v/v) formaldehyde and < 5 % (v/v) methanol.

The reagent is sufficient for 1000 tests when used in the recommended Lyse/No-wash procedure and for 500 tests when used in the recommended Lyse/Wash procedure. The number of tests can differ when using a different protocol.

7 Evidence of deterioration

Avoid contamination of reagents. In case of component 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.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
H341	Suspected of causing genetic defects.
H350	May cause cancer.
H371	May cause damage to organs.
H373	May cause damage to organs through prolonged or repeated exposure.

8.4 Precautions

P201	Obtain special instructions before use.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
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

Dilute CyLyse™ FX (10x concentrate) with deionized water at room temperature (1 volume of concentrated solution with 9 volumes of deionized water).

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 - Lyse/No-wash procedure:

1. Stain whole blood samples following instructions in the Sysmex CyFlow™ antibody reagents IFU.
2. Add 500 µL of 10-fold diluted CyLyse™ FX per 50 µL of whole blood and vortex gently.
3. Incubate for 10-15 minutes at room temperature (18-28 °C) in the dark.
4. Analyse sample immediately using flow cytometer.
5. If the sample is not analysed immediately after staining, store it at 18-28 °C in the dark and analyse it within 6 hours.
6. Resuspend cells by briefly vortexing prior to flow cytometry analysis.

13.2 Manual sample preparation procedure - Lyse/Wash procedure:

1. Stain whole blood samples following instructions in the Sysmex CyFlow™ antibody reagents IFU.
2. Add 1 mL of 10-fold diluted CyLyse™ FX per 50 µL of whole blood and vortex gently.
3. Incubate for 10-15 minutes at room temperature (18-28 °C) in the dark.
4. Centrifuge tubes for 5 minutes at 300 g and remove the supernatant by decanting.
5. Resuspend the cell pellet in a sufficient volume of PBS appropriate for your flow cytometer.
6. Analyse the sample immediately or store it at 2-8 °C in the dark and analyse it within 24 hours.
7. Resuspend cells by briefly vortexing prior to flow cytometry analysis.

13.3 Automated sample preparation procedure:

CyLyse™ FX 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 5×10^6 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].

Sysmex Partec GmbH recommends using the Lyse/Wash procedure due to a better cell separation. However, the Lyse/Wash procedure may lead to a non-specific loss of cells and may not be suitable for absolute cell count determination [33,34].

Results for the Lyse/No-wash procedure may vary depending on the analysis platform. In case of excessive debris in the FSC/SSC plot, the usage of a backbone monoclonal antibody (e.g. CD45) is advised. It is recommended to validate the Lyse/No-wash procedure for your application and flow cytometer to account for example for an increased background fluorescence [33].

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.

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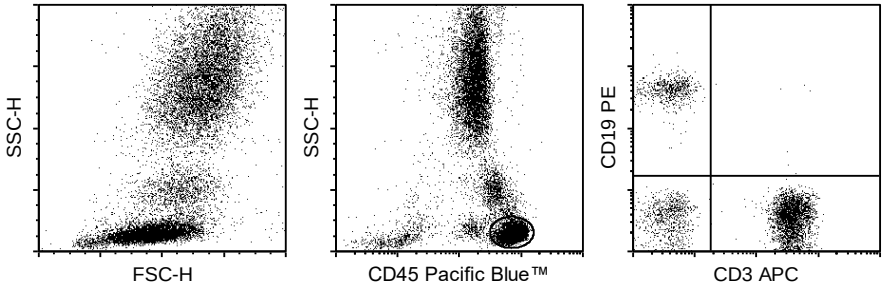
16 Representative data

The following representative data was obtained using human peripheral whole blood stained with Sysmex CyFlow™ antibody reagents (CD3 APC, CD19 PE, and CD45 Pacific Blue™) and treated with CyLyse™ FX. 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	Componentes: Dietilenglicol 20–30 %; Formaldehído < 15 %; Metanol < 5 % Composição: Dietilenglicol 20–30 %; Formaldehyd < 15 %; Metanol < 5 %	Component information for various Latin-American countries
LOT	Batch code	IVD	In vitro diagnostic medical device	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 Toxicologicas de Curitiba: 0800410148	Safety information for various Latin-American countries
	Use-by date		Temperature limit		
	Consult instructions for use		Keep away from sunlight	Información del product: Solución de lisado con fijador para citometría de flujo. Informações sobre o produto: Solução de lise com fixador para citometria de fluxo.	Product information for various Latin-American countries
CE	CE mark	UDI	Unique device identifier	**PELIGRO** H302: Nocivo en caso de ingestión. H315: Provoca irritación cutánea. H317: Puede provocar una reacción alérgica en la piel. H319: Provoca irritación ocular grave. H335: Puede irritar las vías respiratorias. H341: Se sospecha que provoca defectos genéticos. H350: Puede provocar cáncer. H371: Puede provocar daños en los órganos. H373: Puede provocar daños en los órganos tras exposiciones prolongadas o repetidas. P201: Solicitar instrucciones especiales antes del uso. P260: No respirar el polvo/el humo/el gas/la niebla/los vapores/el aerosol. 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. H315: Provoca irritação à pele. H317: Pode provocar reações alérgicas na pele. H319: Provoca irritação ocular grave. H335: Pode provocar irritação das vias respiratórias. H341: Suspeito de provocar defeitos genéticos. H350: Pode provocar câncer. H371: Pode provocar danos aos órgãos. H373: Pode provocar danos aos órgãos por exposição repetida ou prolongada. P201: Obtenha instruções específicas antes da utilização. P260: Não inale as névoas/vapores/aerossóis. 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
UKCA	UKCA mark	UK RP	UK Responsible Person		
CONCENTRATE	Concentrated reagent	Danger	Signal word: Danger		

19 Date of issue or revision

Rev.: 005
Rev. date: 24-08-2023
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