Instructions For Use

Identification of the IVD reagent

CyFlow™ CD45 Pacific Orange™

REF AU422566

IVD CE

Safety Data Sheet for this product is available at www.sysmex-partec.com/services.

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1 Specification

Specificity	Human CD45
Fluorochrome	Pacific Orange™
Clone	2D1
Host / Isotype	Mouse / IgG1
Content	100 tests, 1 mL
Usage	10 µL per test

2 Intended use

IVD **For In Vitro Diagnostic Use.**

The CyFlow™ CD45 Pacific Orange™ monoclonal antibody is intended for qualitative in vitro diagnostic use to identify cells expressing the human CD45 antigen in anticoagulated whole blood using flow cytometry analysis. The CyFlow™ CD45 Pacific Orange™ monoclonal antibody is intended for use by trained laboratory and healthcare professionals in hospitals and clinical laboratories for both manual sample preparation and automated use with a sample preparation system. The CyFlow™ CD45 Pacific Orange™ monoclonal antibody can be used to aid in the diagnosis and classification of disease in patient populations by clinical laboratories.

3 Principle of the examination method

This method is based on specific binding of monoclonal antibody to the target antigen expressed on the cell surface or intracellular cell compartment. Specific monoclonal antibody bound to an antigen is conjugated with a fluorochrome, which is excited by the corresponding laser beam from the appropriately equipped flow cytometer. Subsequent emission of light from the particular fluorochrome is collected and analyzed by a flow cytometer in a dedicated fluorescence detector. Differences in cell fluorescence intensity enable separation of cell subsets based on the expression of the analyzed antigen.

Staining of cells expressing the target antigen is accomplished by incubating a suspension of cells with the monoclonal antibody reagent followed by red blood cells lysis and cell wash to remove unbound monoclonal antibody reagent, when appropriate. Stained cells are subjected to analysis by a flow cytometer.

4 Storage and shelf life after first opening

1. Storage:

Store the reagent at 2-8 ˚C in the dark. Do not freeze or expose to light. Do not use after the expiration date stated on the label.

2. Shelf life after first opening:

Avoid reagent exposure to direct light or freezing conditions after opening. Store at 2-8 ˚C in the dark. Close lid tight to avoid spills and to maintain the vial free of condensation.
CyFlow™ CD45 Pacific Orange™ monoclonal antibody retains its performance characteristics after having been placed into use for a minimum of 725 days.

5 Components

Mouse monoclonal antibody (clone 2D1, isotype IgG1) against human CD45 antigen labelled with Pacific Orange™ fluorochrome.

The reagent is provided in stabilizing phosphate buffered saline (PBS) solution, pH ≈7.4, containing 0.09% (w/v) sodium azide and 0.2% (w/v) BSA.

6 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. For serious incidents please contact the manufacturer and a competent authority.

7 Precautions and warnings

The reagent contains sodium azide (NaN_3) which is highly toxic in pure form. However, the concentration in the reagent is not hazardous. When disposing the reagent, follow the applicable local regulations.

The reagent contains BSA. Handle as though capable of transmitting infection.

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

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

8 Additional required equipment

Instrument requirement:	Flow cytometer equipped with appropriate light source, filters and detectors in order to detect light scatter and the appropriate fluorescence, and equipped with appropriate software for data acquisition and analysis.
	Vortex mixer
	Centrifuge (including rotor system designed for up to 500 g)
	Optional: Sample preparation system (e.g. Sysmex Sample Preparation System PS-10)
Required material:	Material necessary for the collection of whole blood
	Disposable test tubes for staining of blood samples
	Pipettes with disposable tips for 10, 100 and 1000 µL
	Adequate personal protective equipment
	Commercial lysing solution, for example: CyLyse™ LV, Ref. No.: BL215283 (CE IVD)
	Phosphate buffered saline (PBS), pH 7.4
	Fixation solution, for example: 2% paraformaldehyde solution in PBS (optional)

9 Reagent preparation

The reagent is ready to use. The content of a vial (1 mL) is sufficient for 100 tests.

10 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.

11 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 an anticoagulant. Keep the tube with the blood specimen at room temperature (18-28 °C). Before use, gently mix. For the best results, the use of fresh blood sample is recommended. The time between collection and analysis should not be longer than 24 hours.

12 Examination procedure

12.1 Staining

1. Add 10 µL of CyFlow™ monoclonal antibody reagent to a test tube.

2. Add 100 µL of blood specimen to the tube and vortex gently.

3. Incubate for 20-30 minutes at room temperature (18-28 °C) in the dark.

4. Perform red blood cell lysis. Follow the instructions of the lysing solution manufacturer. For example, if you wish to use CyLyse™ LV, add 2 mL of 10X diluted CyLyse™ LV lysing solution per 100 µL of whole blood and vortex gently.

5. Incubate for 10-15 minutes at room temperature (18-28 °C) in the dark.

6. Centrifuge tubes for 5 minutes at 300 g and remove supernatant by decanting.

7. Resuspend cell pellet with 2 mL of PBS.

8. Centrifuge tubes for 5 minutes at 300 g and remove supernatant by decanting.

9. For subsequent analysis on a flow cytometer, resuspend the cell pellet in a sufficient volume of PBS appropriate for your flow cytometer.

10. For later analysis, resuspend the cells in a fixative instead. It is recommended to use 2% paraformaldehyde solution in PBS. Store the fixed sample at 2-8 °C in the dark and analyze within 24 hours.

11. Vortex tube thoroughly and carefully to reduce cell aggregation prior to flow cytometry analysis.

12.2 Sample analysis

12.2.1 Internal quality control

Laser alignment and regular instrument calibration using fluorescent particles is strongly advised in order to achieve precise and reproducible measurements. Each laboratory shall perform instrument Quality Control according to the instruction given by the supplier of the instrument.

12.2.2 Flow cytometry

Analyse the samples using a flow cytometer with appropriate configuration. Before sample analysis minimise debris and ensure that the populations of interest are included in the gating to avoid improper results.

12.3 Data acquisition and analysis

Acquire and analyse the data using a flow cytometer with appropriate configuration.

Always acquire cell light scatter parameters: forward-scatter (FSC) and side-scatter (SSC).

Refer to manufacturer's cytometer specifications for fluorescence detectors in which emission of fluorescence of antibody stained CD45+ events is collected according to the emission characteristics of the fluorochrome.

Emission spectra of some fluorochromes overlap. Compensation of measured data may be required prior to analysis.

Representative data of CyFlow™ CD45 Pacific Orange™ performed on whole blood and gated on leukocytes are shown in Figure 1.

Figure 1: Representative data of CyFlow™ CD45 Pacific Orange™ analyzed with flow cytometer equipped with violet laser (laser excitation 405 nm).

13 Interpretation of results

Abnormal numbers of cells expressing this antigen or aberrant expression levels of the antigen will be expected in some disease states. It is important to understand the normal expression pattern for the investigated antigen and its relationship to expression of other relevant antigens in order to perform appropriate analysis.

14 Control procedure

Sysmex Partec GmbH recommends running a blood control sample from a normal adult subject (healthy donor) daily or a commercially available whole blood control to optimize flow cytometer settings and as a quality control check of the system.

15 Performance characteristics

15.1 Analytical Specificity

The monoclonal antibody 2D1 was assigned to human CD45 antigen during the International Workshop and Conference on Human Leucocyte Differentiation Antigens.

HLDA III – WS Code 792

15.2 Analytical Sensitivity

Analytical sensitivity for qualitative flow cytometry reagent is the ability to separate positive (+) cells from negative (-) background and was evaluated by titration of the antibody reagent.

The bottled concentration of CyFlow™ CD45 Pacific Orange™ (120 µg/mL) is sufficient to allow a separation of positive cells from background either in a specimen with normal or abnormal antigen expression expected in certain pathological states.

15.3 Representative data

To determine the performance of staining with CyFlow™ CD45 Pacific Orange™ reagent, the percentage of positive CD45 cells gated on CD3+ lymphocytes was determined using two reagent lots stained with five blood samples from healthy donors in five replicates per lot and sample.

CD45+ cells (gated on CD3+ lymphocytes)

	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
Mean (%)	99.82	99.80	99.92	99.90	99.93
SD	0.12	0.13	0.03	0.07	0.04
CV (%)	0.12	0.13	0.03	0.07	0.04

Table 1: Summary of obtained results

16 Limitations

The test is intended for professional healthcare users in clinical laboratories performing flow cytometry analysis.

The single antibody reagent can provide only limited diagnostic information. Using combinations of reagents can provide more information than using reagents individually and multicolor analysis using relevant combinations of reagents is highly recommended. Laboratories should identify reagent combinations that best suit their needs based on the properties of each single antibody reagent and the presence of markers in normal and abnormal samples. Such reagent combinations should then be validated by the laboratory based on that application and intended use.

Blood samples from non-healthy persons may exhibit abnormal values of positive cells.

Hemolysis may indicate improper storage conditions that may affect product performance in terms of lysability. Therefore hemolyzed samples should be excluded.

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

Red blood cells from abnormal patients (e.g. polycitemic patients) may be resistant to lysis using lysing solutions.

Reagent performance data was collected with K3 EDTA-treated blood. Other anticoagulants may affect the reagent performance.

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

Sysmex Partec GmbH recommends using the Lyse/Wash procedure. The Lyse/No-wash procedure should be used with caution and only if the monoclonal antibody has been validated for the intended assay.

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

Product labelled as CE IVD is intended for In Vitro Diagnostic use in laboratories outside the USA.

17 Literature references

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18 Contact

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20 Symbols

REF	Reference number		Use-by date
IVD	In vitro diagnostic medical device		Content sufficient for <n> tests
CE	CE mark	CLONE	Clone of hybridoma cells used to produce monoclonal antibody
LOT	Batch code		Temperature limit
	Manufacturer		Keep away from sunlight
	Caution		Consult instructions for use
UK RP	UK Responsible Person	UKCA	UKCA mark
Producto químico no clasificado como peligroso según GHS. Produto químico não classificado como perigoso de acordo com a ABNT NBR 14725-2.	Statement for various Latin-American countries	UDI	Unique device identifier