DryFlowEx PNH High-Sensitivity Assay Kit

25 tests | Cat. No. **ED7750**

IVD CE

Instructions for Use (EN)

Version: ED7750_IFU_v1_EN

Date of Issue: 22-03-2023

Symbols used in the device labeling

IVD	In Vitro diagnostic medical device		Temperature limit
CE	CE marking of conformity		Keep away from sunlight
	Manufacturer		Keep Dry Keep away from rain
UDI	Unique Device Identifier		Caution
	Consult instructions for use		Do not re-use
	Contains sufficient for <n> tests	TUBE	Contains <n> tubes for single use test
REF	Catalogue number	CONC 10×	Concentrated solution (10x)
LOT	Batch code	CONTENTS	Contents
	Use by date	UK CA	UKCA mark

1. Intended Purpose

DryFlowEx PNH High-Sensitivity Assay Kit is intended for high sensitivity detection and enumeration of glycosyl-phosphatidyl-inositol (GPI)-deficient cells in human whole blood by flow cytometry.

What is detected and/or measured

The device DryFlowEx PNH High-Sensitivity Assay Kit detects and enumerates glycosyl-phosphatidyl-inositol (GPI)-deficient cells (PNH clones) as a percentage of:

- CD59 dim or CD59- cells from all erythrocytes (CD235a+)
- CD59 dim or CD59- cells from all reticulocytes (CD235a+CD71+)
- CD14-, CD157- and GPI anchor- cells from all monocytes (CD45+CD64+)
- CD24-, CD157- and GPI anchor- cells from all neutrophil granulocytes (CD45+CD15+)

Device function

The device is intended for diagnosis and monitoring of patients suffering, or suspected of suffering Paroxysmal Nocturnal Hemoglobinuria (PNH) and related disorders [(1)].

Context of a physiological or pathological state

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder resulting as a consequence of the non-malignant clonal expansion of cells with somatic mutation of Phosphatidylinositol Glycan Anchor Biosynthesis Class A (PIGA) gene. Mutations of the PIGA gene result in the inability to express glycosyl-phosphatidyl-inositol (GPI)-anchored cell surface proteins.

The device is intended to detect GPI-deficient neutrophil granulocytes and monocytes [(1)], together with complete (Type III) and partial (Type II) GPI-deficient erythrocytes [(2,3,4,5,6)] for evaluation of the PNH clone size.

Furthermore, the device detects GPI-deficient reticulocytes (immature erythrocytes) in patients receiving blood transfusions when PNH erythrocytes are difficult to delineate [(7)].

Type of assay

Not automated

Quantitative

Type of specimen required

Human anticoagulated peripheral whole blood specimen (EDTA, heparin, citrate) [(1)]

Testing population

Patients with:

- laboratory markers of hemolysis, when other more common causes of hemolysis have been excluded,
- unexplained thromboses in young age,
- diagnosed thromboses in an unusual site,
- inherited or acquired aplastic anemia (AA),
- myelodysplastic syndrome (MDS),
- unexplained cytopenia in whom AA or MDS are differential diagnostic considerations [1]

2. Intended user

The device is intended for professional laboratory use only. Not for near-patient testing or self-testing.

Requirements on qualification

Intended user shall have state-of-the-art expertise in flow cytometry analysis of human cells, standard laboratory techniques including pipetting skills, safe and proper handling of specimens derived from the human body.

Intended user shall be compliant with standard EN ISO 15189 or other national provisions, where applicable.

3. Test principle

The test principle is based on the detection of GPI anchor and GPI-anchored proteins on the surface of human blood cells. Monoclonal antibodies and recombinant proaerolysin used in the test are labeled with different fluorochromes which are excited by a laser beam from a flow cytometer during acquisition of a stained blood specimen. Subsequent fluorescence (light emission) from each fluorochrome present on an acquired blood cell is collected and analyzed by the instrument. Fluorescence intensity is directly proportional to the antigen expression density in a cell allowing for separation of different cell subsets.

4. Reagent(s) provided

Contents

The device DryFlowEx PNH High-Sensitivity Assay Kit, sufficient for examination of 25 patients, is provided with the following reagents:

PNH High-Sensitivity Assay (25 pouches). Each pouch consists of 1 color-coded (Cyan strip) capped single-use tube **PNH WBC 7-color** (ED7750-1) and 1 color-coded (Red strip) capped single-use tube **PNH RBC 3-color** (ED7750-2), containing premixed combinations of fluorochrome-labeled reagents dried with the stabilizing ingredients as a layer at bottom of the test tubes (12 x 75 mm), see Table 1 and 2.

Lysing Solution ED7750-3 (1 bottle) containing 15 ml of concentrated (10X) formaldehyde-based buffered solution.

PNH Compensation Set ED7750-4 (1 pouch) containing 10 capped single-use tubes, each containing single fluorochrome-labeled reagent dried with the stabilizing ingredients as a layer at the bottom of the tube (12 x 75 mm).

> **CAUTION**: PNH Compensation Set is intended for the compensation setup only. Single fluorochrome-labeled reagents (see Table 1 and Table 2) allow easy and accurate compensation procedure.

Composition

Table 1 Description of the PNH WBC 7-color active ingredients

Antigen	Fluorochrome	Clone	Isotype
GPI anchor (Proaerolysin)	Alexa Fluor®488	N/A	N/A
CD157	PE	SY11B5	IgG1
CD45	PerCP-Cy™5.5	2D1	IgG1
CD64	PE-Cy™7	10.1	IgG1
CD24	APC	SN3	IgG1
CD14	APC-Cy™7	MEM-15	IgG1
CD15	Pacific Blue™	MEM-158	IgM

Table 2 Description of the PNH RBC 3-color active ingredients

Antigen	Fluorochrome	Clone	Isotype
CD235a	FITC	JC159	IgG1
CD59	PE	MEM-43	IgG2a
CD71	APC	MEM-75	IgG1

5. Materials required but not provided

Deionized water (Reagent-grade)

Phosphate buffered saline (1x PBS), pH 7.2 – 7.4

Flow Cytometry Compensation Particles (Spherotech SPHERO™ COMPtrol Kit, Cat. No. CMIgP-50-3K or equivalent compensation particles)

6. Equipment required

Automatic pipette with disposable tips (100 µl – 5 ml) for pipetting specimen and reagents

Liquid dispenser or pipette with disposable tips (2 ml) for dispensing erythrocyte lysing solution

Vortex mixer

Conical polypropylene centrifuge tubes (15 ml or 50 ml) for specimen preparation

Centrifuge with appropriate rotor adaptors for 12 x 75 mm round bottom tubes

Flow cytometer with three laser excitation sources (488 nm, ~635 nm and 405 nm), detectors for scatters, optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 3

Table 3 Spectral characteristic of fluorochromes used in the device

Flurochrome	Excitation [nm]	Emission [nm]
Alexa Fluor® 488	488	520
FITC	488	525
PE	488	576
PerCP-Cy™5.5	488	695
PE-Cy™7	488	780
APC	630 – 640	660
APC-Cy™7	630 – 640	780
Pacific Blue™	405	455

NOTICE: The device was tested on flow cytometers BD FACSCanto™ II (BD Biosciences), BD FACSLyric™ (BD Biosciences), Navios EX (Beckman Coulter), DxFLEX (Beckman Coulter).

7. Storage and handling

Store at 20-30 °C.

Avoid prolonged exposure to light.

Keep dry.

CAUTION: Moisture sensitive product. Do not open the foil pouch until the first use.

See Section 10 Procedure (Preparation of reagent(s) provided) for information about the storage conditions and stability of working solutions (where applicable).

8. Warnings, precautions and limitations of use

GHS Hazard Classification

WARNING: Lysing solution (ED7750-3) contains formaldehyde (CAS No. 50-00-0) and methanol (CAS No. 67-56-1) in concentrations classified as hazardous.

Label elements	Signal word
	**Danger**
H-phrases	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 the kidneys through prolonged or repeated exposure if swallowed. H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled.
P-phrases	P201: Obtain special instructions before use. P260: Do not breathe vapours. P264: Wash hands and exposed parts of the body thoroughly after handling. P280: Wear protective gloves/eye protection/face protection. P301+P312: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P302+P352: IF ON SKIN: Wash with plenty of water and soap. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308+P313: IF exposed or concerned: Get medical advice/attention. P314: Get medical advice/attention if you feel unwell. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.

Consult Safety Data Sheet (SDS) available on the product page at www.exbio.cz for the full information on the risks posed by chemical substances and mixtures contained in the Product and how they should be handled and disposed.

Biological Hazard

Human biological samples and blood specimens and any materials coming into contact with them are always considered as infectious materials.

Use personal protective and safety equipment to avoid contact with skin, eyes and mucous membranes.

Follow all applicable laws, regulations and procedures for handling and disposing of infectious materials.

Evidence of deterioration

Normal appearance of the reagent provided is a transparent dried layer at the bottom of the tube. Do not use the reagent if you observe any change in appearance, for example presence of moisture inside the tube.

Limitation of use

Do not use after the expiry date stated on the product labels.

Do not re-use test tubes.

9. Specimen

Use venous peripheral blood collected in specimen receptacle classified as a medical device, with EDTA, Heparin, or ACD (Acid Citrat Dextrose) anticoagulant [(2)].

Blood specimen in the collection tube must be stored at room temperature. Do not refrigerate.

Only use non-treated specimen. Do not use pre-lysed, washed or diluted specimen.

Process the blood specimen no later than 48 hours after collection [(2)].

10.Procedure

Preparation of reagent(s) provided

PNH High-Sensitivity Assay

No reagent preparation is necessary, supplied in test tubes for single use only.

Lysing Solution

Dilute (10X) Lysing solution with deionized water according to the manufacturer´s instructions. Diluted (1X) Lysing solution is stable for 1 month when stored in a liquid dispenser or closed container at room temperature.

Preparation of materials required but not provided

Compensation particles

Prepare working solution of flow cytometry compensation particles according to manufacturer´s instructions.

Compensation setup

Acquire Compensation Set tubes using the same flow cytometer set-up, prior to the analysis of PNH RBC 3-color and PNH WBC 7-color stained tubes.

CAUTION: PNH RBC 3-color and PNH WBC 7-color compensation setup procedures differ in type of specimen preparation and sample staining.

PNH RBC 3-color compensation tubes (Red strip)

1. Add SPHERO™ COMPtrol Kit or equivalent compensation particles into the bottom of each single-color compensation tube.
2. Vortex and incubate for 20 minutes at room temperature in the dark.
3. Add 4 ml of 1X PBS to each compensation tube. Centrifuge for 5 minutes at 300×g.
4. Discard supernatant without disturbing the compensation particles and add 0.1 ml of 1X PBS to each compensation tube.
5. Set voltages on fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interfere with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.
6. Acquire the stained compensation tubes immediately using flow cytometer.
7. Calculate PNH RBC 3-color compensation matrix either in cytometer software developed by manufacturer or software dedicated for offline cytometry data analysis. Use this compensation matrix for all test tubes of this lot of PNH RBC 3-color.

CAUTION: Once set for the specific PNH RBC 3-color lot, do not change fluorescent detectors settings in order to retain the same compensation matrix acquisition settings and compensation results.

PNH WBC 7-color compensation tubes (Cyan strip)

1. Add 50 µl deionized water into the bottom of each single-color compensation tube and vortex vigorously for 7-10 seconds.
2. Add 100 µl of peripheral whole blood to each single-color compensation tube

and vortex vigorously.

3. Incubate for 20 minutes at room temperature in the dark.
4. Add 2 ml of dilute (1X) Lysing Solution to each compensation tube.
5. Incubate for 10 minutes at room temperature in the dark.
6. Centrifuge for 5 minutes at 300×g, discard supernatant and resuspend the cell pellet in 2 ml of 1X PBS.
7. Centrifuge for 5 minutes at 300×g, discard supernatant and resuspend the cell pellet in 0.2 ml of 1X PBS.
8. Set voltages on fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interfere with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.
9. Acquire the stained compensation tubes immediately using flow cytometer.
10. Calculate PNH WBC 7-color compensation matrix either in cytometer software developed by manufacturer or software dedicated for offline cytometry data analysis. Use this compensation matrix for all test tubes of this lot of PNH WBC 7-color.

    CAUTION: Once set for the specific PNH WBC 7-color lot, do not change fluorescent detectors settings in order to retain the same compensation matrix acquisition settings and compensation results.

Specimen preparation

Detection and differentiation of PNH clones in erythrocytes using PNH RBC 3-color tube requires specimen preparation prior to the staining procedure.

NOTICE: Before processing the specimen, ensure that the cytometer has been properly set up.

1. Label a polypropylene conical tube with the identification of examined blood specimen.
2. Pipette 10 µl of well-mixed blood specimen to the bottom of the labeled conical tube.
3. Dilute blood specimen 1:100 with 1 ml of 1X PBS and mix by hand swaying for 5 seconds.

   CAUTION: Classic form of PNH is dominated by intravascular hemolysis. Prior to diluting blood specimen, refer to RBC counts from hematology analyzer in order

to achieve RBC count in diluted blood specimen in the range of 3 – 5 × 10^7 / ml of diluted blood and adjust dilution factor as required in order to acquire sufficient count of RBCs in flow cytometer.

4. Proceed to Specimen staining procedure immediately after specimen dilution.

Detection of GPI-deficient cells in neutrophil granulocytes and monocytes using PNH WBC 7-color tube requires no specimen preparation prior to the staining procedure.

Specimen staining – PNH RBC 3-color tube (Red strip)

1. Label PNH RBC 3-color tube with the identification of examined blood specimen.
2. Pipette 50 µl of well-mixed diluted blood specimen into the bottom of the PNH RBC 3-color tube.

   CAUTION: Avoid pipetting blood on the side of the test tube. If blood smear or droplet remains on the side of the tube, it will not be stained with the reagent and the test results can be invalid.
3. Vortex vigorously for 7-10 seconds.
   CAUTION: Shortening the vortex time may affect the test results.
4. Incubate PNH RBC 3-color tube for 20 minutes at room temperature in the dark.
5. Add 4 ml of 1X PBS to PNH RBC 3-color tube.
6. Centrifuge the PNH RBC 3-color tube for 5 minutes at 300× g.
7. Discard supernatant without disturbing the cell pellet and add 0.5 ml of 1X PBS to the PNH RBC 3-color tube.
8. Vortex shortly to resuspend the cell pellet.

Acquire the stained sample using flow cytometer. If the stained sample will not be acquired immediatelly, cap the test tube, store at 2-8 °C in the dark and analyze within 2 hours.

CAUTION: Disrupt cell aggregates in the stained sample by sliding the test tube against the tube rack immediately before acquisition on the flow cytometer. Excessive amount of RBC aggregates may affect the test results.

Specimen staining – PNH WBC 7-color tube (Cyan strip)

1. Label PNH WBC 7-color tube with the identification of examined blood specimen.

2. Add 50 µl of deionized water to the PNH WBC 7-color test tube. Vortex vigorously for 7-10 seconds.

   CAUTION: Shortening the vortex time may affect the test results.

3. Pipette 100 µl of well-mixed blood specimen into the bottom of the PNH WBC 7-color tube and vortex vigorously.

   CAUTION: Avoid pipetting blood on the side of the test tube. If blood smear or droplet remains on the side of the tube, it will not be stained with the reagent and the test results can be invalid.

4. Incubate for 20 minutes at room temperature in the dark.
5. Add 2 ml of 1X working erythrocyte Lysing Solution to PNH WBC 7-color tube.
6. Incubate for 10 minutes at room temperature in the dark.
7. Centrifuge the PNH WBC 7-color tube for 5 minutes at 300× g.
8. Discard supernatant without disturbing the cell pellet and add 2 ml of 1X PBS to the test tube.
9. Centrifuge the PNH WBC 7-color tube for 5 minutes at 300× g.
10. Discard supernatant without disturbing the cell pellet and add 0.2 ml of 1X PBS to the test tube.
11. Vortex shortly to resuspend the cell pellet.

Acquire the stained sample using flow cytometer. If the stained sample will not be acquired immediatelly, cap the test tube, store at 2-8 °C in the dark and analyze within 24 hours.

Flow cytometry analysis

The flow cytometer selected for use with the device DryFlowEx PNH High-Sensitivity Assay Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturers instructions.

If not maintained properly the flow cytometer may produce false results.

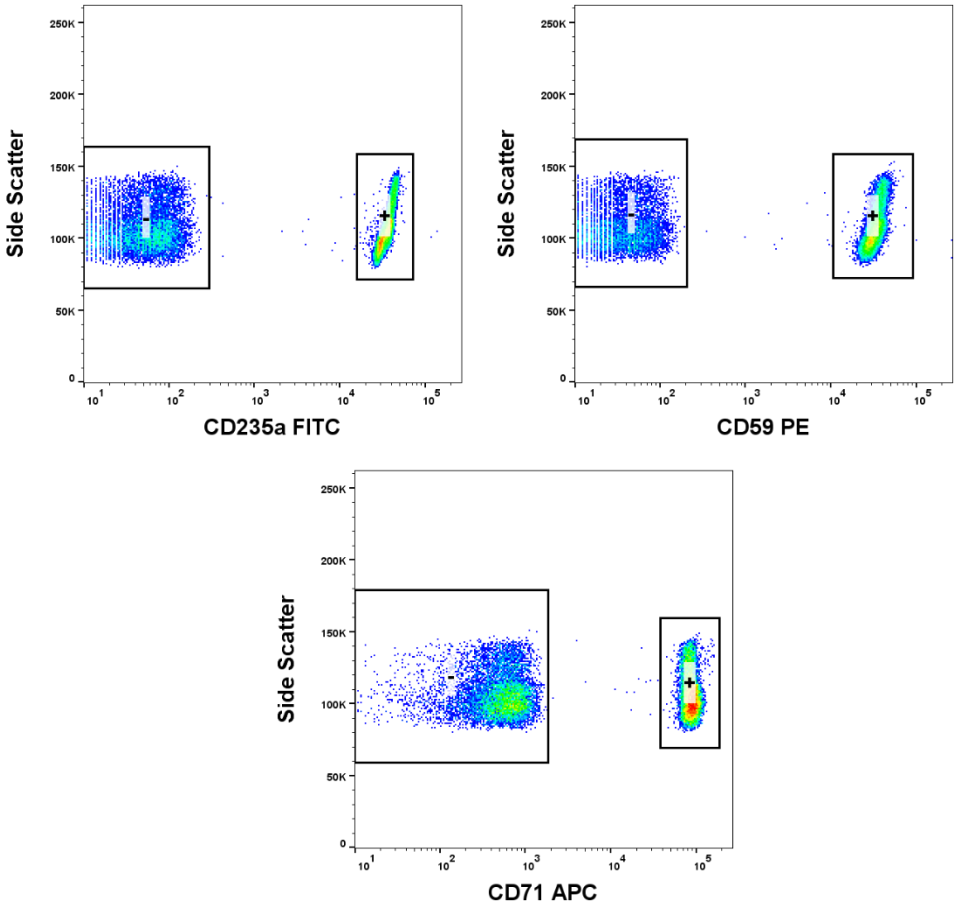
Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section 6 Equipment required.

For measured data analysis, it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

Analysis of PNH RBC 3-color compensation tubes (Red strip)

Visualize non-compensated data for each compensation tube in a side-scatter (SSC) versus “fluorochrome to be compensated” dot-plot. Set the gates for positive (+) and negative (-) cytometry compensation particles as shown in Figure 1.

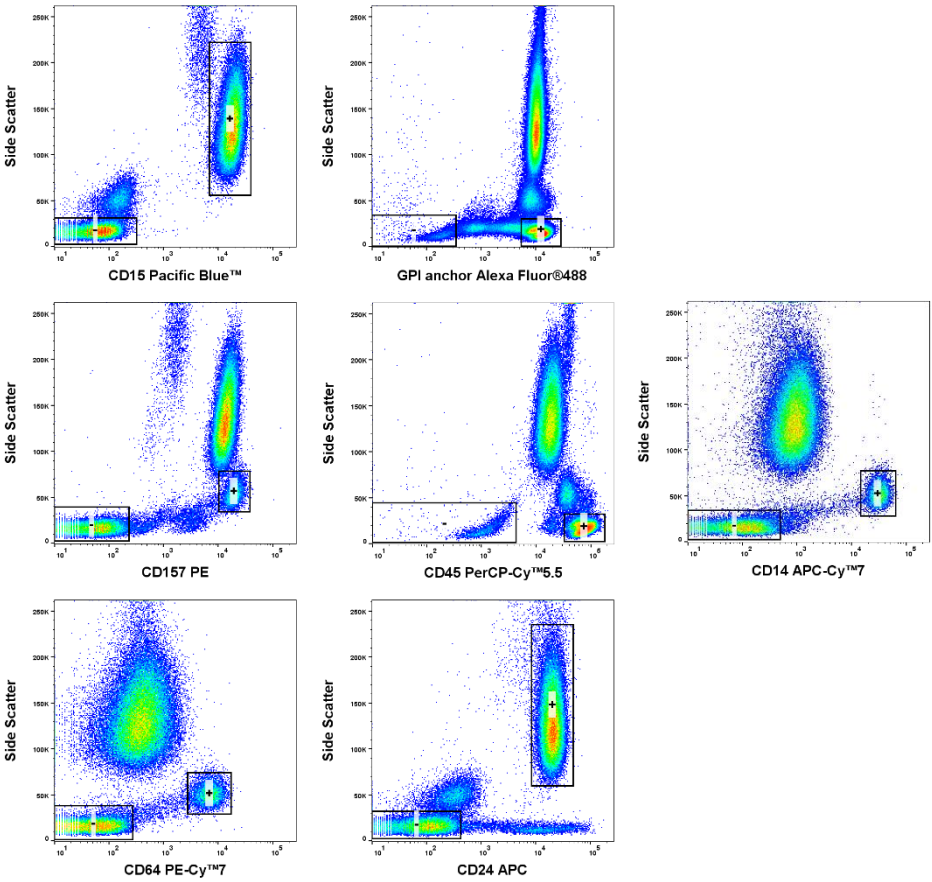
Figure 1 Identification of positive (+) and negative (-) cytometry compensation particles in compensation tubes (data acquired on BD FACSCanto™ II).



Analysis of PNH WBC 7-color compensation tubes (Cyan strip)

Visualize non-compensated data for each compensation tube in a side-scatter (SSC) versus “fluorochrome to be compensated” dot-plot. Set the gates for the most positive (+) and the most negative (-) populations as shown in Figure 2.

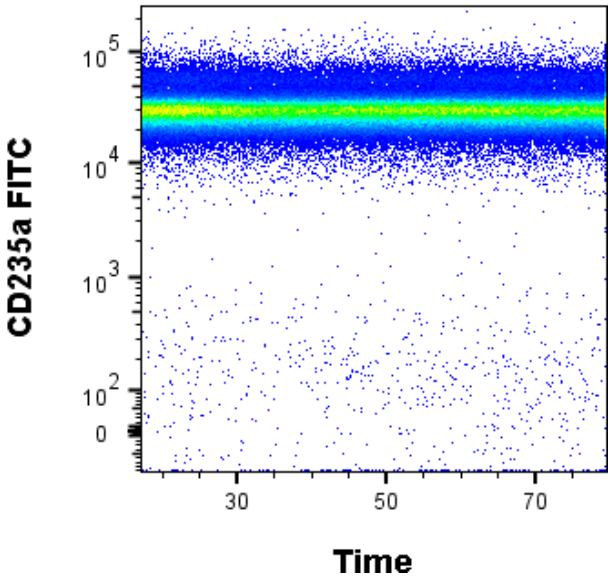
Figure 2 Identification of the most positive (+) and the most negative (-) events in compensation tubes (data acquired on BD FACSCanto™ II).



PNH RBC 3-color tube (Red strip)

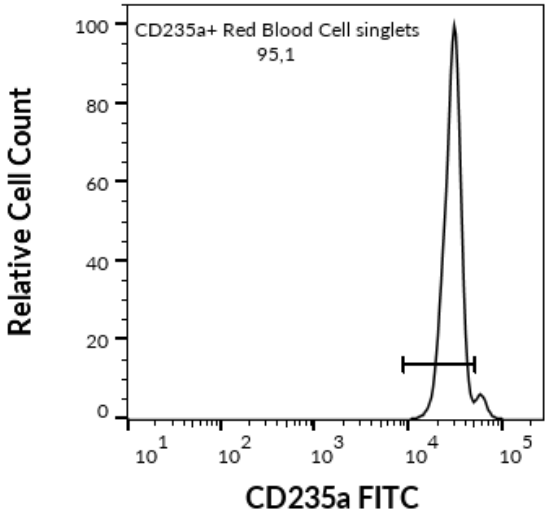
Due to low reticulocyte count in diluted blood specimen, aquire 500.000 – 1.500.000 erythrocyte events for analysis. Acquisition of ≥ 500.000 events results in long acquisition times. This may affect antibody-antigen binding complex equilibrium and the decrease of CD235a FITC fluorescence. Always monitor the stability of fluorescence intensity over the acquisition time (Figure 3).

Figure 3 All acquired events in a dot-plot CD235a FITC vs. Time (data acquired on BD FACSCanto™ II).



Visualize compensated data as a histogram where the X-axis represents fluorescence intensity in FITC channel. Set “CD235a+ RBC singlets” gate (Figure 4).

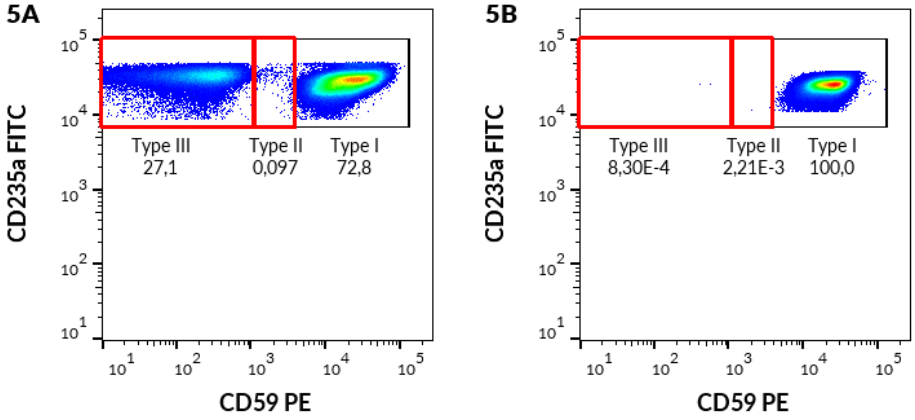
Figure 4 Delineation of CD235a+ RBC singlets (data acquired on BD FACSCanto™ II).



Erythrocytes

Visualize CD235a+ RBC singlets in a dot-plot CD59 PE versus CD235a FITC. Separate events into three populations using three appropriate gates (Figure 5) and calculate the percentage of events in the regions of Type I, Type II and Type III.

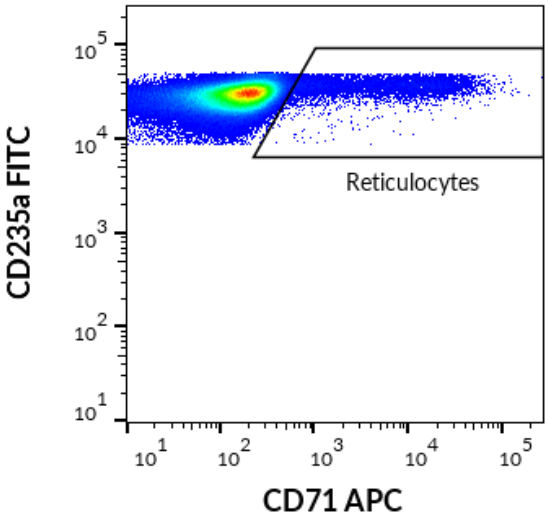
Figure 5 CD235a+ RBC singlets in a dot-plot CD59 PE vs. CD235a FITC (data acquired on BD FACSCanto™ II).
A) patient with PNH clone; B) healthy donor



Reticulocytes

Visualize CD235a+ RBC singlets in a dot-plot CD71 APC versus CD235a FITC and separate CD71+ reticulocytes (Figure 6).

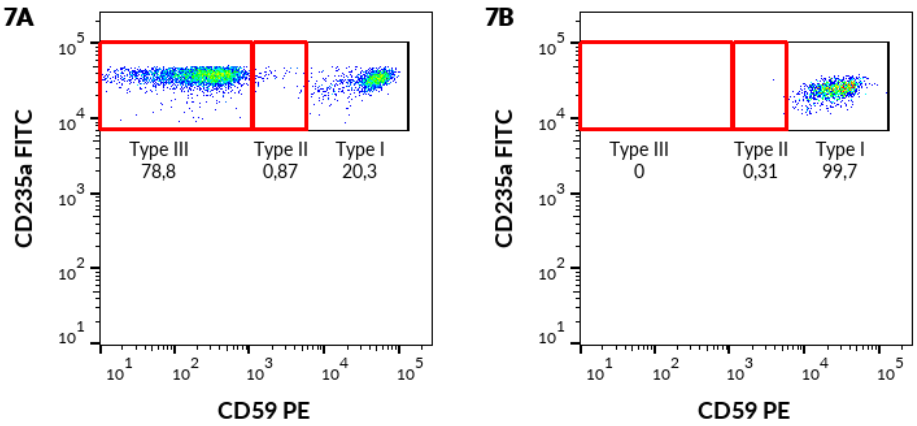
Figure 6 CD235a+ RBC singlets in a dot-plot CD71 APC vs. CD235a FITC. Delineation of CD71+ reticulocytes (data acquired on BD FACSCanto™ II).



Visualize CD71+ reticulocytes in a dot-plot CD59 PE versus CD235a FITC. Separate events into three populations using three appropriate gates (Figure 7) and calculate the percentage of events in the regions of Type I, Type II and Type III.

Figure 7 CD71+ Reticulocytes in a dot-plot CD59 PE vs. CD235a FITC (data acquired on BD FACSCanto™ II).

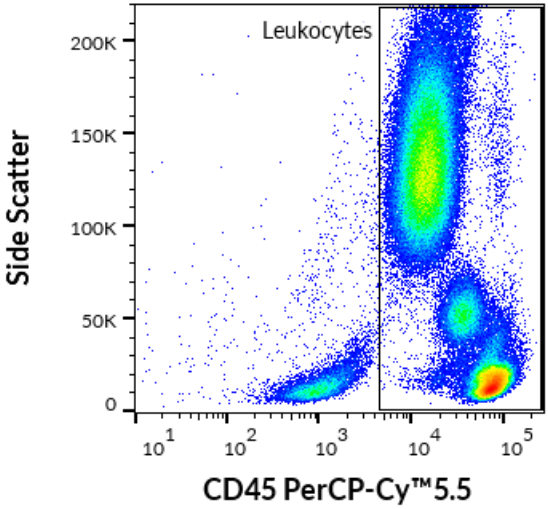
A) patient with PNH clone; B) healthy donor



PNH WBC 7-color tube (Cyan strip)

Acquire at least 200.000 events for analysis. Visualize compensated data in s dot-plot side-scatter versus fluorescence intensity in PerCP-Cy™ 5.5. Set CD45+ leukocytes gate as shown in Figure 8.

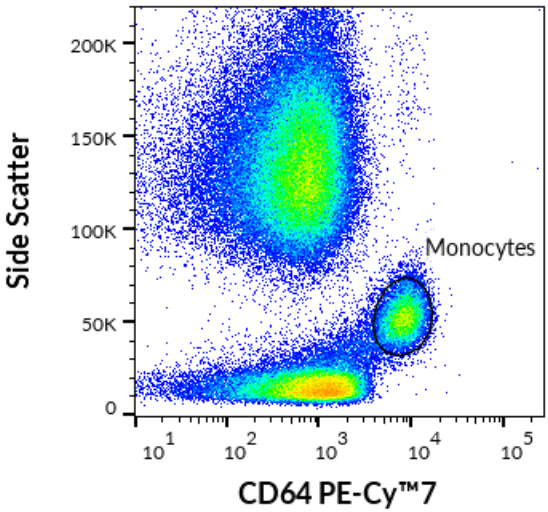
Figure 8 Delineation of CD45+ Leukocytes (data acquired on BD FACSCanto™ II).



Monocytes

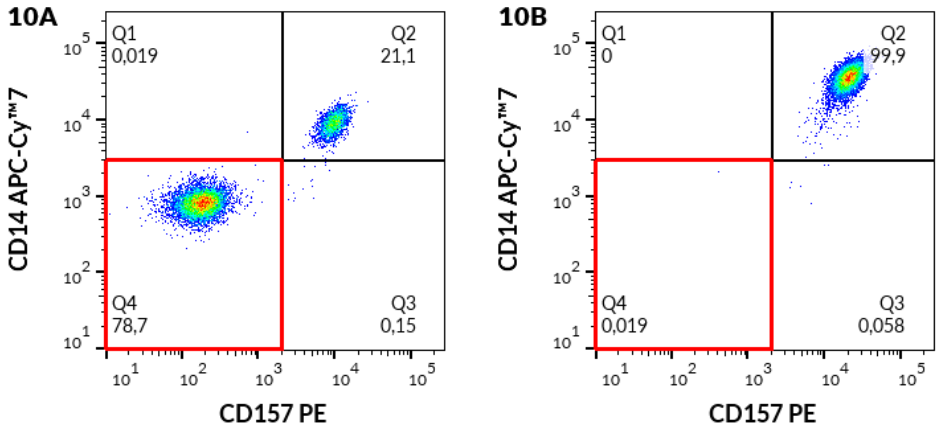
Visualize CD45+ leukocytes in a dot-plot side-scatter versus CD64 PE-Cy™7 and delimit CD64+ monocytes as shown in Figure 9.

Figure 9 Delineation of CD64+ Monocytes from Leukocytes (data acquired on BD FACSCanto™ II).



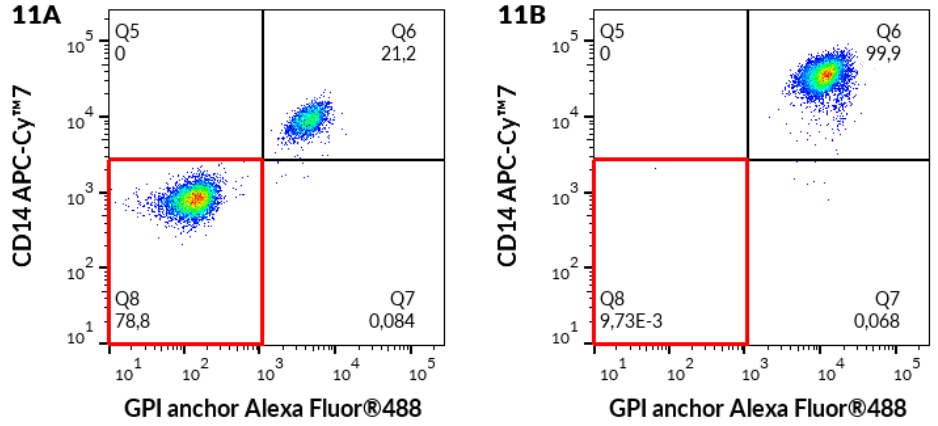
Visualize CD64+ monocytes in a dot-plot CD157 PE versus CD14 APC-Cy™7 (Figure 10). Set appropriate gates and calculate the percentage of CD157-CD14- population in Q4 quadrant.

Figure 10 CD64+ Monocytes in a dot-plot CD157 PE vs. CD14 APC-Cy™7 (data acquired on BD FACSCanto™ II).
A) patient with PNH clone; B) healthy donor



Then visualize the same CD64+ monocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) versus CD14 APC-Cy™7 (Figure 11). Set appropriate gates and calculate the percentage of GPI anchor- CD14- population in Q4 quadrant.

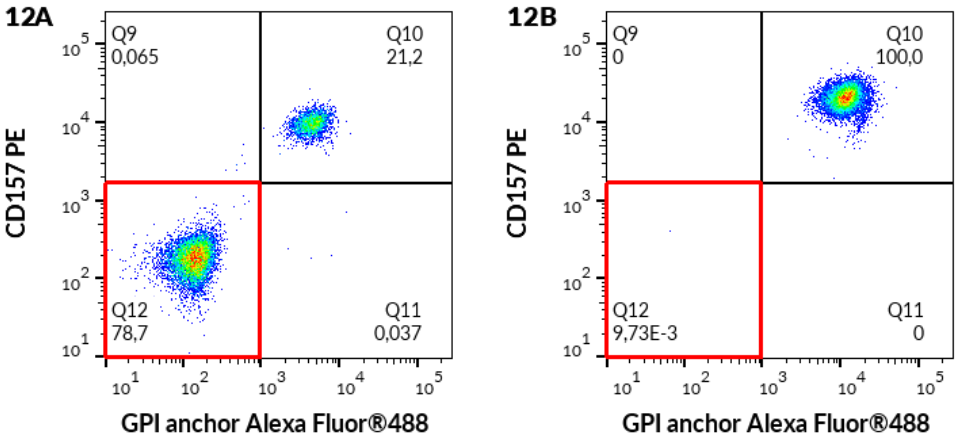
Figure 11 CD64+ Monocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) vs. CD14 APC Cy™7 (data acquired on BD FACSCanto™ II).
A) patient with PNH clone; B) healthy donor



Then visualize the same CD64+ monocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) versus CD157 PE (Figure 12). Set appropriate gates and calculate the percentage of GPI anchor- CD157- population in Q4 quadrant.

Figure 12 CD64+ Monocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) vs. CD157 PE (data acquired on BD FACSCanto™ II).

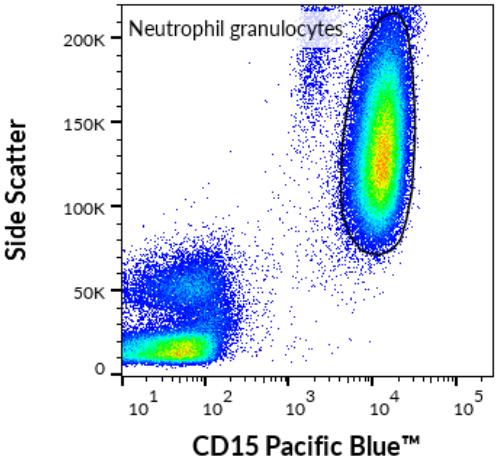
A) patient with PNH clone; B) healthy donor



Neutrophil Granulocytes

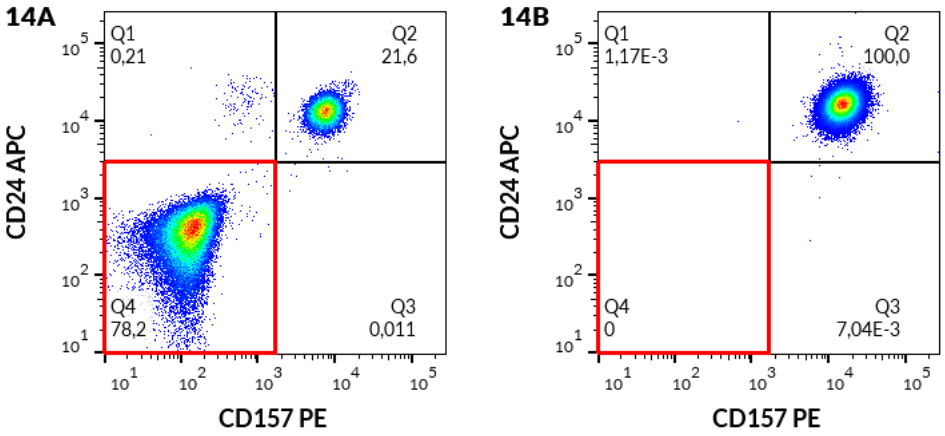
Visualize CD45+ leukocytes in a dot-plot side-scatter versus CD15 Pacific Blue™ and separate CD15+ neutrophil granulocytes as shown in Figure 13.

Figure 13 Delineation of CD15+ Neutrophil granulocytes from Leukocytes (data acquired on BD FACSCanto™ II).



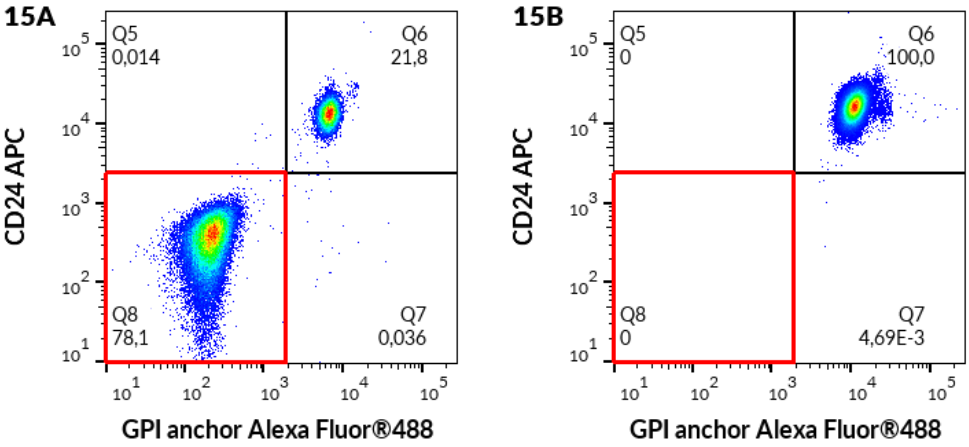
Visualize CD15+ neutrophil granulocytes in a dot-plot CD157 PE versus CD24 APC as shown in Figure 14. Set appropriate gates and calculate the percentage of CD157- CD24- population in Q4 quadrant.

Figure 14 CD15+ Neutrophil granulocytes in a dot-plot CD157 PE vs. CD24 APC (data acquired on BD FACSCanto™ II).
A) patient with PNH clone; B) healthy donor



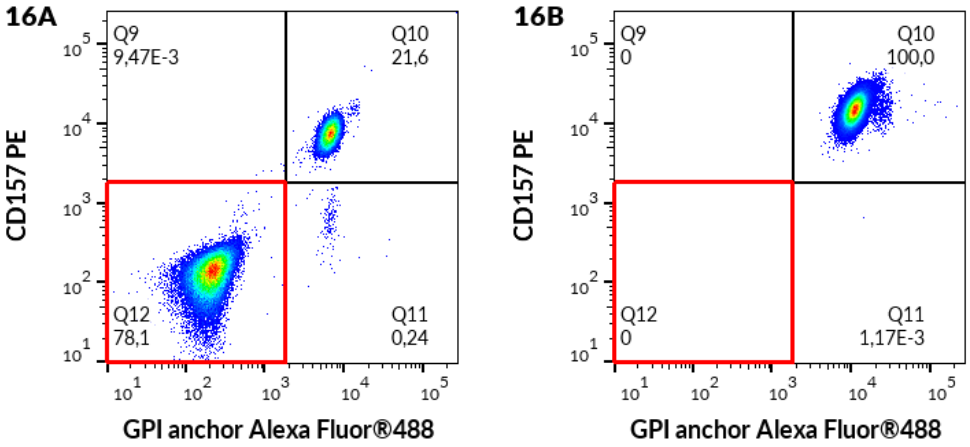
Then visualize the same CD15+ neutrophil granulocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) versus CD24 APC, set appropriate gates and calculate the percentage of GPI anchor- CD24- population in Q4 quadrant as shown in Figure 15.

Figure 15 CD15+ Neutrophil granulocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) vs. CD24 APC (data acquired on BD FACSCanto™ II).
A) patient with PNH clone; B) healthy donor



Then visualize the same CD15+ neutrophil granulocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) versus CD157 PE, set appropriate gates and calculate the percentage of GPI anchor- CD157- population in Q4 quadrant as shown in Figure 16.

Figure 16 CD15+ Neutrophil granulocytes in a dot-plot Proaerolysin Alexa Fluor® 488 (GPI anchor) vs. CD157 PE (data acquired on BD FACSCanto™ II).
A) patient with PNH clone; B) healthy donor



Calculation and interpretation of analytical results

Enumerate the percentage of GPI deficient (having PNH phenotype) cells, see Table 4.

Table 4 PNH clone phenotypes

Parent cell population		PNH phenotype according to the gating strategy
PNH RBC 3-color Tube	Erythrocytes (Type III)	CD59- CD235a+ (Fig. 5)
	Erythrocytes (Type II)	CD59 dim CD235a+ (Fig. 5)
	Reticulocytes (Type III)	CD59- CD235a+CD71+(Fig. 7)
	Reticulocytes (Type II)	CD59 dim CD235a+CD71+(Fig. 7)
PNH WBC 7-color Tube	Monocytes	CD14- CD157- CD64+ (Fig. 10)
		CD14- GPI anchor- CD64+ (Fig. 11)
		CD157- GPI anchor- CD64+ (Fig. 12)
	Neutrophil Granulocytes	CD24- CD157- CD15+ (Fig. 14)
		CD24- GPI anchor- CD15+ (Fig. 15)
		CD157- GPI anchor- CD15+ (Fig. 16)

Table 5 Results interpretation

Limit of detection (cut-off) for the WBC and RBC tubes reported as frequency of parent (%), calculated from 100 measurements of n=25 normal patient samples on n=4 different cytometer platforms				
PNH phenotype	Cytometer			
	BD FACS Lyric™	BD FACS Canto™ II	Beckman Coulter NAVIOS EX	Beckman Coulter DX Flex
PNH RBC 3-color tube				
CD59- Type II and Type III RBCs	0.005	0.002	0.029	0.049
CD59- Type II and Type III Reticulocytes	0.240	0.320	0.388	0.562
PNH WBC 7-color Tube				
CD157- CD14- Monocytes	0.20	0.19	0.14	0.30
GPI anchor- CD14- Monocytes	0.08	0.04	0.10	0.17
GPI anchor- CD157- Monocytes	0.07	0.06	0.04	0.03
CD157- CD24- Neutrophil Granulocytes	0.02	0.02	0.06	0.03
GPI anchor- CD24- Neutrophil Granulocytes	0.03	0.03	0.02	0.02
GPI anchor- CD157- Neutrophil Granulocytes	0.01	0.01	0.01	0.01

GPI deficiency reporting algorithm rules

1. In patients with GPI-deficient cell population frequency **lower** than Cut-off value (Table 5), results are to be reported as: "**granulocytes, monocytes, RBCs and reticulocytes show normal expression of GPI-linked antigens. No PNH clones detected**" [(1)].

2. In patients with GPI-deficient cell population frequency **higher** than Cut-off value (Table 5), results are to be reported as**: "granulocytes, monocytes, RBCs, or reticulocytes show partial or complete GPI- deficiency". PNH clones detected.**

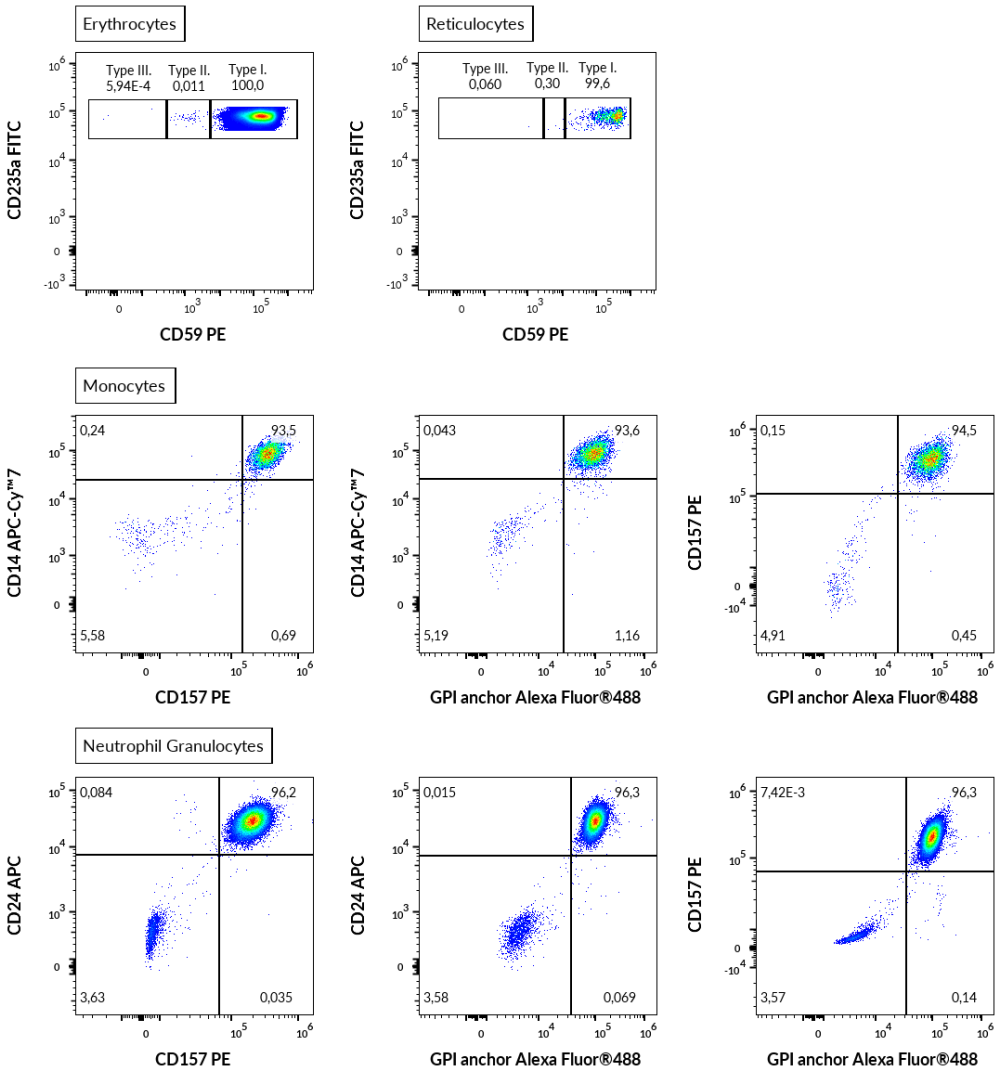
   CAUTION: Clinical laboratory must establish its own limit of detection (LOD)/Cut-off values from a set of normal patient samples when using a different type and/or brand of cytometer than specified in Tables 7-10 (see section 11. Analytical performance/ Limit of detection / Assay Cut-off).

3. In most PNH cases all WBC target cell populations show the presence of PNH clone[(4, 6, 7, 8)]. WBC PNH clones appear clustered and less scattered than random double negative events.

4. In some cases, presence of a PNH clone may be detected in WBC tube, while not being detected in a RBC tube as shown in **Fig. 17**. In this case, presence of a PNH clone has to be reported as per **GPI deficiency reporting algorithm rule 2**.

5. If any PNH clone is detected, always report percentage for all PNH clone phenotypes (Table 5) from their parent cell population. Monocytes may show larger PNH clone size than neutrophil granuclocytes[(2)].

Figure 17 Example of a case with presence of a PNH clone in a WBC tube, while not being detected in a RBC tube (data acquired on Beckman Coulter DxFLEX).



11.Analytical performance

Specificity

Proaerolysin Alexa Fluor® 488 is fluorescently labeled variant of bacterial aerolysin that specifically binds to GPI anchors of surface membrane proteins in human cells [1, 2, 5, 8].

The antibody SY11B5 recognizes an extracellular epitope on CD157 antigen of the CD157 antigen expressed mainly on monocytes and granulocytes. Specificity of the antibody has been confirmed by HCDM Council (HLDA X workshop).

The antibody 2D1 recognizes all leukocyte isoforms of human CD45 (Leukocyte Common Antigen). Specificity of the antibody has been confirmed by HCDM Council (HLDA III workshop).

The antibody 10.1 recognizes of human CD64 antigen, that is expressed on monocytes. Specificity of the antibody has been confirmed by HLDA workshops (HLDA III: WS Code M-250 workshop).

The antibody SN3 reacts with CD24 antigen, expressed by granulocytes. Specificity of the antibody has been confirmed by HLDA workshop (HLDA IV: WS Code B 136; HLDA V: WS Code B CD24.7)

The antibody MEM-15 reacts with CD14, a GPI (glycosylphosphatidylinositol)-linked extracellular membrane glycoprotein expressed on monocytes. Specificity of the antibody has been confirmed by HCDM Council (HLDA III: WS Code M 252; HLDA IV: WS Code M 113; HLDA IV: WS Code NL 90; HLDA IV: WS Code T 53; HLDA V: WS Code M MA086; HLDA VI: WS Code M MA94 workshop).

The antibody MEM-158 reacts with CD15, strongly expressed on the surface of granulocytes. Specificity of the antibody has been confirmed by HCDM Council (HLDA VI: WS Code AS A053 workshop).

The antibody JC159 recognizes an epitope of the extracellular portion of CD235a (glycophorin A), a sialoglycoprotein expressed on early erythroblasts, late erythroblasts, erythroblasts and mature erythrocytes.

The antibody MEM-43 reacts with well defined epitope on CD59 (Protectin), (GPI)-anchored glycoprotein expressed on the surface of all hematopoietic cells. Specificity of the antibody has been confirmed by HLDA workshop (HLDA IV: WS Code NL 705; HLDA V: WS Code AS S013; HLDA V: WS Code BP BP345; HLDA V: WS Code T T-103 workshop).

The antibody MEM-75 reacts with an extracellular epitope of CD71 antigen expressed on immature reticulocytes. Specificity of the antibody has been confirmed by HLDA workshop (HLDA IV: WS Code A 45; HLDA V: WS Code T T-165 workshop).

Accuracy

Accuracy of the method was determined as a comparison of the device DryFlowEx PNH High-Sensitivity Assay Kit with an accredited clinical laboratory in-house method by parallel staining of 13 patients with confirmed presence of PNH phenotype. Linear regression analysis parameters are provided in Table 6.

Table 6 Linear regression analysis for relative counts of GPI deficient cell populations (PNH phenotypes) in patients with confirmed presence of PNH phenotypes (comparison of the device DryFlowEx PNH High-Sensitivity Assay kit with an accredited clinical laboratory in-house method (a cocktail of single color conjugated antibodies from different manufacturers and analysed using BD FACSCanto™ II))

Lymphocyte Subset	n	Slope	Intercept	R^2	Range [%]
CD59- CD235a+ Type III erythrocytes	13	0.99	-0.026	1.00	1.28 – 83.79
CD59- CD235a+ Type III reticulocytes	13	0.99	-0.384	1.00	5.97 – 97.78
CD59- CD235a+ Type II erythrocytes	13	1.00	-0.059	1.00	0.13 – 89.92
CD59- CD235a+ Type II reticulocytes	13	0.98	0.141	1.00	0.33 – 74.67
CD157- GPI anchor- CD64+ monocytes	13	1.00	0.060	1.00	2.07 – 99.95
CD157- GPI anchor- CD15+ neutrophils	13	0.99	0.294	1.00	0.80 – 99.82
CD14- GPI anchor- CD64+ monocytes	13	Not determined			2.04 – 99.96
CD14- CD157- CD64+ monocytes	13	Not determined			2.17 – 99.96
CD24- CD157- CD15+ neutrophils	13	Not determined			0.80 – 99.83
CD24- GPI anchor- CD15+ neutrophils	13	Not determined			0.81 – 99.80

Limit of detection / Assay Cut-off

Limit of detection (LOD) has been determined for each target population (see Table 5) as a mean value of results from 25 healthy blood donors increased by addition of three standard deviations from the mean for 4 different flow cytometer platforms and expressed as Assay Cut-off in Table 7, 8, 9, and 10.

CAUTION: Clinical laboratory must establish its own limit of detection (LOD)/Cut-off values from a set of normal patient samples when using a different type and/or brand of cytometer than specified in Tables 7-10.

Table 7 DryFlowEx PNH High-Sensitivity Assay Kit Cut-off values for each PNH phenotype together with PNH phenotype incidence and LOQ acquired on BD FACSLyric™ flow cytometer.

PNH phenotype	BD FACSLyric™					
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**Cut-off (Mean + 3*SD)**	**LOQ (Mean + 10*SD)**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)						
CD59- Type II and Type III RBCs	25	0.003	0.001	**5 – 48 events per 1,000,000 events** (mean 25 events)	**0.005 %**	**0.012 %**
CD59- Type II and Type III Reticulocytes	25	0.054	0.061	**0 – 5 events per 3,000 Reticulocytes** (mean 2 events)	**0.240 %**	**0.660 %**
WBC Tube (200.000 events acquired)						
CD157- CD14- Monocytes	25	0.076	0.041	**2 - 24 events per 10,000 Monocytes** (mean 8 events)	**0.20 %**	**0.49 %**
GPI anchor- CD14- Monocytes	25	0.021	0.018	**0 - 5 events per 10,000 Monocytes** (mean 2 events)	**0.08 %**	**0.20 %**
GPI anchor- CD157- Monocytes	25	0.014	0.020	**0 - 4 events per 10,000 Monocytes** (mean 1 events)	**0.07 %**	**0.21 %**
CD157- CD24- Neutrophil Granulocytes	25	0.006	0.006	**0 - 20 events per 100,000 Neutrophils** (mean 5 events)	**0.02 %**	**0.07 %**
GPI anchor- CD24- Neutrophil Granulocytes	25	0.006	0.008	**0 - 29 events per 100,000 Neutrophils** (mean 6 events)	**0.03 %**	**0.09 %**
GPI anchor- CD157- Neutrophil Granulocytes	25	0.002	0.002	**0 - 8 events per 100,000 Neutrophils** (mean 2 events)	**0.01 %**	**0.02 %**

Table 8 DryFlowEx PNH High-Sensitivity Assay Kit Cut-off values for each PNH phenotype together with PNH phenotype incidence and LOQ acquired on BD FACSCanto™ II flow cytometer.

PNH phenotype	BD FACSCanto™ II					
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**Cut-off (Mean + 3*SD)**	**LOQ (Mean + 10*SD**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)						
CD59- Type II and Type III RBCs	25	0.0006	0,0004	**1 – 12 events per 1,000,000 events** (mean 6 events)	**0.002 %**	**0.004 %**
CD59- Type II and Type III Reticulocytes	25	0.0657	0.0847	**0 – 5 events per 1,000 Reticulocytes** (mean 1 events)	**0.320 %**	**0.913 %**
WBC Tube (200.000 events acquired)						
CD157- CD14- Monocytes	25	0.085	0.035	**2 - 16 events per 10,000 Monocytes** (mean 8 events)	**0.19 %**	**0.43 %**
GPI anchor- CD14- Monocytes	25	0.086	0.096	**0 - 3 events per 10,000 Monocytes** (mean 1 events)	**0.04 %**	**0.10 %**
GPI anchor- CD157- Monocytes	25	0.084	0.019	**0 - 7 events per 10,000 Monocytes** (mean 1 events)	**0.06 %**	**0.20 %**
CD157- CD24- Neutrophil Granulocytes	25	0.004	0.052	**0 - 17 events per 100,000 Neutrophils** (mean 5 events)	**0.02 %**	**0.06 %**
GPI anchor- CD24- Neutrophil Granulocytes	25	0.006	0.010	**0 - 32 events per 100,000 Neutrophils** (mean 6 events)	**0.03 %**	**0.10 %**
GPI anchor- CD157- Neutrophil Granulocytes	25	0.002	0.002	**0 - 8 events per 100,000 Neutrophils** (mean 2 events)	**0.01 %**	**0.02 %**

Table 9 DryFlowEx PNH High-Sensitivity Assay Kit Cut-off values for each PNH phenotype together with PNH phenotype incidence and LOQ acquired on Beckman Coulter Navios EX flow cytometer.

PNH phenotype	Beckman Coulter Navios EX					
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**Cut-off (Mean + 3*SD)**	**LOQ (Mean + 10*SD**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)						
CD59- Type II and Type III RBCs	25	0.007	0.007	**4 – 236 events per 1,000,000 events** (mean 60 events)	**0.029 %**	**0.081 %**
CD59- Type II and Type III Reticulocytes	25	0.087	0.100	**0 – 6 events per 1,000 Reticulocytes** (mean 1 events)	**0.388 %**	**1.092 %**
WBC Tube (200.000 events acquired)						
CD157- CD14- Monocytes	25	0.062	0.027	**0 - 23 events per 10,000 Monocytes** (mean 6 events)	**0.14 %**	**0.33 %**
GPI anchor- CD14- Monocytes	25	0.024	0.006	**0 - 10 events per 10,000 Monocytes** (mean 2 events)	**0.10 %**	**0.28 %**
GPI anchor- CD157- Monocytes	25	0.007	0.011	**0 - 6 events per 10,000 Monocytes** (mean 1 events)	**0.04 %**	**0.12 %**
CD157- CD24- Neutrophil Granulocytes	25	0.012	0.015	**0 - 43 events per 100,000 Neutrophils** (mean 12 events)	**0.06 %**	**0.16 %**
GPI anchor- CD24- Neutrophil Granulocytes	25	0.005	0.005	**0 - 13 events per 100,000 Neutrophils** (mean 5 events)	**0.02 %**	**0.05 %**
GPI anchor- CD157- Neutrophil Granulocytes	25	0.002	0.002	**0 - 10 events per 100,000 Neutrophils** (mean 2 events)	**0.01 %**	**0.03 %**

Table 10 DryFlowEx PNH High-Sensitivity Assay Kit Cut-off values for each PNH phenotype together with PNH phenotype incidence and LOQ acquired on Beckman Coulter DxFLEX flow cytometer.

PNH phenotype	Beckman Coulter DxFLEX					
	n	Mean [%]	SD [%]	**PNH phenotype incidence**	**Cut-off (Mean + 3*SD)**	**LOQ (Mean + 10*SD**
RBC tube (1.000.000 events acquired; min. 80% singlet RBC events)						
CD59- Type II and Type III RBCs	25	0,015	0.012	**5 – 48 events per 1,000,000 events** (mean 25 events)	**0.049 %**	**0.129 %**
CD59- Type II and Type III Reticulocytes	25	0.106	0.152	**0 – 5 events per 1,000 Reticulocytes** (mean 2 events)	**0.562 %**	**1.626 %**
WBC Tube (200.000 events acquired)						
CD157- CD14- Monocytes	25	0.092	0.068	**0 - 27 events per 10,000 Monocytes** (mean 10 events)	**0.30 %**	**0.77 %**
GPI anchor- CD14- Monocytes	25	0.053	0.040	**0 - 16 events per 10,000 Monocytes** (mean 6 events)	**0.17 %**	**0.46 %**
GPI anchor- CD157- Monocytes	25	0.005	0.009	**0 - 1 events per 10,000 Monocytes** (mean 1 events)	**0.03 %**	**0.10 %**
CD157- CD24- Neutrophil Granulocytes	25	0.010	0.008	**0 - 28 events per 100,000 Neutrophils** (mean 10 events)	**0.03 %**	**0.09 %**
GPI anchor- CD24- Neutrophil Granulocytes	25	0.008	0.006	**0 - 20 events per 100,000 Neutrophils** (mean 8 events)	**0.02 %**	**0.06 %**
GPI anchor- CD157- Neutrophil Granulocytes	25	0.002	0.002	**0 - 5 events per 100,000 Neutrophils** (mean 2 events)	**0.01 %**	**0.02 %**

12.Clinical performance

Patients with GPI deficiency

Clinical data was collected at a clinical site from 19 patients, both healthy (6) and patients with confirmed GPI deficiency (13). Clinical performance was determined as a comparison of the device DryFlowEx PNH High-Sensitivity Assay Kit with an accredited clinical laboratory in-house method (a cocktail of single color conjugated antibodies from different manufacturers and analysed using BD FACSCanto™ II).

GPI deficiency in pacients has been evaluated in regard to the method used (Table 11) by detection of GPI deficient cells (PNH clones).

Table 11 Clinical performance of the device DryFlowEx PNH High-Sensitivity Assay Kit

		GPI deficiency assessment using accredited clinical laboratory in-house method	
		GPI deficiency	**Normal condition**
GPI deficiency assessment using the device ED7750 DryFlowEx PNH High-Sensitivity Assay Kit	**GPI deficiency**	13 patients	0 patients
	Normal condition	0 patients	6 patients

13.Expected values

Percentage of GPI deficient cell populations (PNH phenotypes) in normal healthy patients are expected to be below Cut-off value for each PNH phenotype (Table 5).

CAUTION: Indicated values using the device DryFlowEx PNH High-Sensitivity Assay Kit are intended to be representative only. Each laboratory must establish its own values of limit of detection (cut-off) from the local population of normal donors.

14.Interfering substances and limitations

No interfering substances have been identified, nor tested.

No limitations for use in specific types of diseases, like anemias, have been identified.

GPI deficiency reporting is limited as per the current state-of-the-art published guidelines [6].

15.References

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16.Trademarks

17.Revision History

Version 1, ED7750_IFU_v1

Initial Release

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NOTICE: Any serious incident that has occured in relation to the device shall be reported to the manufacturer and the local competent authority.