DryFlowEx PNH High-Sensitivity Assay (RUO)

25 tests | Cat. No. **ED7787**

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7787_TDS_v4_EN

Date of Issue: 13-03-2026

Symbols used in the product labeling

RUO	Research Use Only		Keep Dry Keep away from the rain
	Manufacturer		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		
	Temperature limit		
	Keep away from sunlight		

Description

The product is for Research Use Only. Diagnostic or therapeutic applications are strictly forbidden.

DryFlowEx PNH High-Sensitivity Assay (RUO) detects and counts glycosyl-phosphatidyl-inositol (GPI)-deficient cells.

Specification

DryFlowEx PNH High Sensitivity Assay (RUO) contains a PNH WBC 7-color tube to stain leukocytes and a PNH RBC 3-color tube to stain red blood cells.

The lysing solution is formulated to fix and lyse cells stained with the PNH WBC 7-color tube.

PNH Compensation Set is used for the preparation of compensation tubes to compensate signals of PNH WBC 7-color and PNH RBC 3-color tubes.

Reagent(s) provided

Contents

The product DryFlowEx PNH High-Sensitivity Assay (RUO), sufficient for 25 tests, 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** (ED7787-1) and 1 color-coded (Red strip) capped single-use tube **PNH RBC 3-color** (ED7787-2), containing premixed combinations of fluorochrome-labeled reagents dried with the stabilizing ingredients as a layer at the bottom of the test tubes (12 x 75 mm), see Tables 1 and 2.

Lysing Solution ED7787-3 (1 bottle) containing 15 ml of concentrated (10X) formaldehyde-based buffered solution.

PNH Compensation Set ED7787-4 (1 pouch) containing 10 capped single-use tubes, each containing a 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 Tables 1 and 2) enable an 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

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)

Equipment required

Automatic pipette with disposable tips (100 µl – 5 ml) for pipetting specimens 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 characteristics of fluorochromes used in the product

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

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

Warnings, precautions and limitations of use

WARNING: Lysing Solution (ED7787-3) contains formaldehyde (CAS No. 50-00-0), methanol (CAS No. 67-56-1) and 2,2'-oxybisethanol (CAS No. 111-46-6) in concentrations classified as hazardous.

Label elements	Signal word
	**Danger**
H-phrases	H302: Harmful if swallowed. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. H331: Toxic if inhaled. 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. EUH071: Corrosive to the respiratory tract.
P-phrases	P201: Obtain special instructions before use. P260: Do not breathe vapours. P280: Wear protective gloves/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/ attention. P403+P233: Store in a well-ventilated place. Keep container tightly closed.

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, blood specimens, and any materials that come into contact with them are always considered 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

The 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, such as the presence of moisture inside the tube.

Limitations of use

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

Do not reuse test tubes.

Specimen

Use venous peripheral blood collected in specimen receptacle classified as a medical device, with EDTA, Heparin, or ACD (Acid Citrat Dextrose) anticoagulant [(1)].

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

Process the blood specimen within 48 hours of collection. Keep the specimen at laboratory temperature (20–25°C) if it will be processed within 24 hours.
If processing is delayed beyond 24 hours but within 48 hours, refrigerate the specimen [(1)].

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

Bring the reagent to room temperature before use.

The reagent is 10X concentrated and must be diluted with deionized water prior use (1 volume of the concentrated solution and 9 volumes of deionized water).

Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container.

The diluted lysing olution (1X) 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 a working solution of flow cytometry compensation particles according to the manufacturer´s instructions.

Compensation setup

Acquire Compensation Set tubes using the same flow cytometer set-up, before 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 the 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 before stained specimen analysis. Voltage on a PMT detector should be set high enough so that a minimum of negatively stained events interfere with the 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 a flow cytometer.
7. Calculate PNH RBC 3-color compensation matrix either in the cytometer software developed by the manufacturer or software dedicated to 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 before stained specimen analysis. Voltage on a PMT detector should be set high enough so that a minimum of negatively stained events interfere with the 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 a flow cytometer.
10. Calculate PNH WBC 7-color compensation matrix either in the cytometer software developed by the manufacturer or software dedicated to 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 the PNH RBC 3-color tube requires specimen preparation before 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 the examined blood specimen.
2. Pipette 10 µl of well-mixed blood specimen to the bottom of the labeled conical tube.
3. Dilute the 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 the Specimen staining procedure immediately after specimen dilution.

Detection of GPI-deficient cells in neutrophils and monocytes using the PNH WBC 7-color tube requires no specimen preparation before the staining procedure.

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

1. Label the PNH RBC 3-color tube with the identification of the 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 the 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 a flow cytometer. If the stained sample will not be acquired immediately, 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 6-10 times against the top of 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 the PNH WBC 7-color tube with the identification of the 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 a 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 the 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 a flow cytometer. If the stained sample will not be acquired immediately, 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 product DryFlowEx PNH High-Sensitivity Assay (RUO) shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturer's instructions.

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

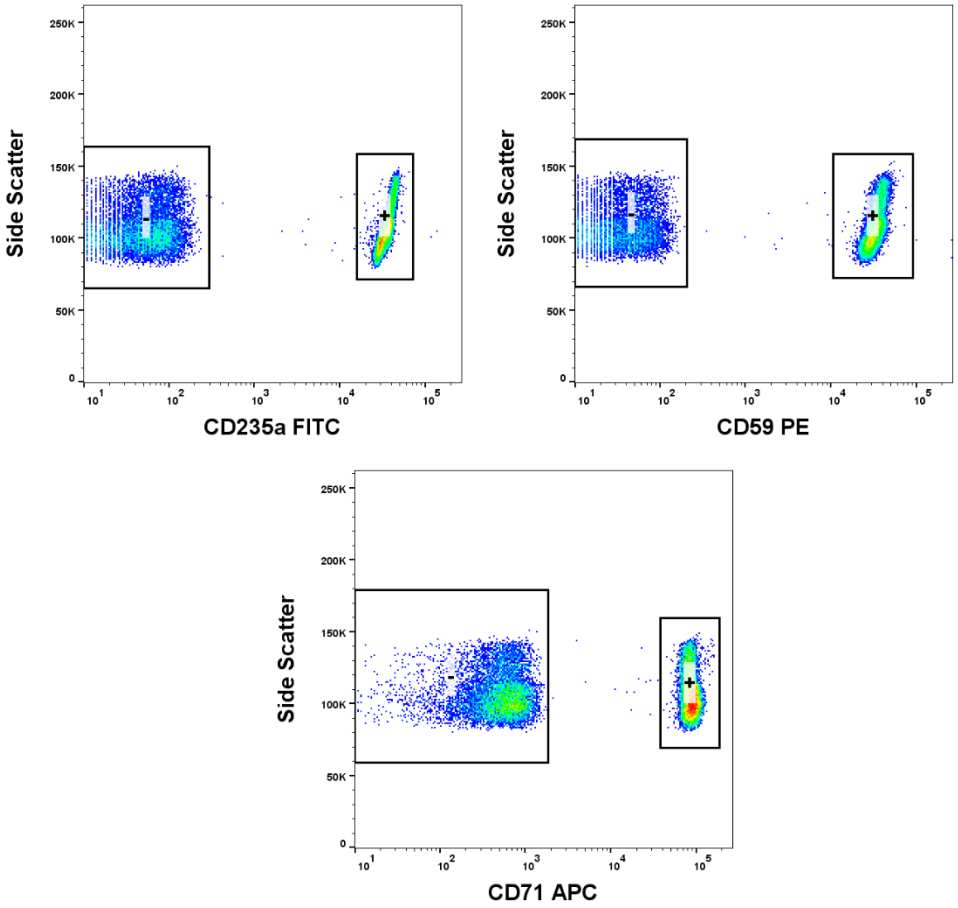
Refer to the manufacturer’s cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section “Equipment required”.

For measured data analysis, it is possible to use cytometer software developed by the manufacturer, or software dedicated to 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.

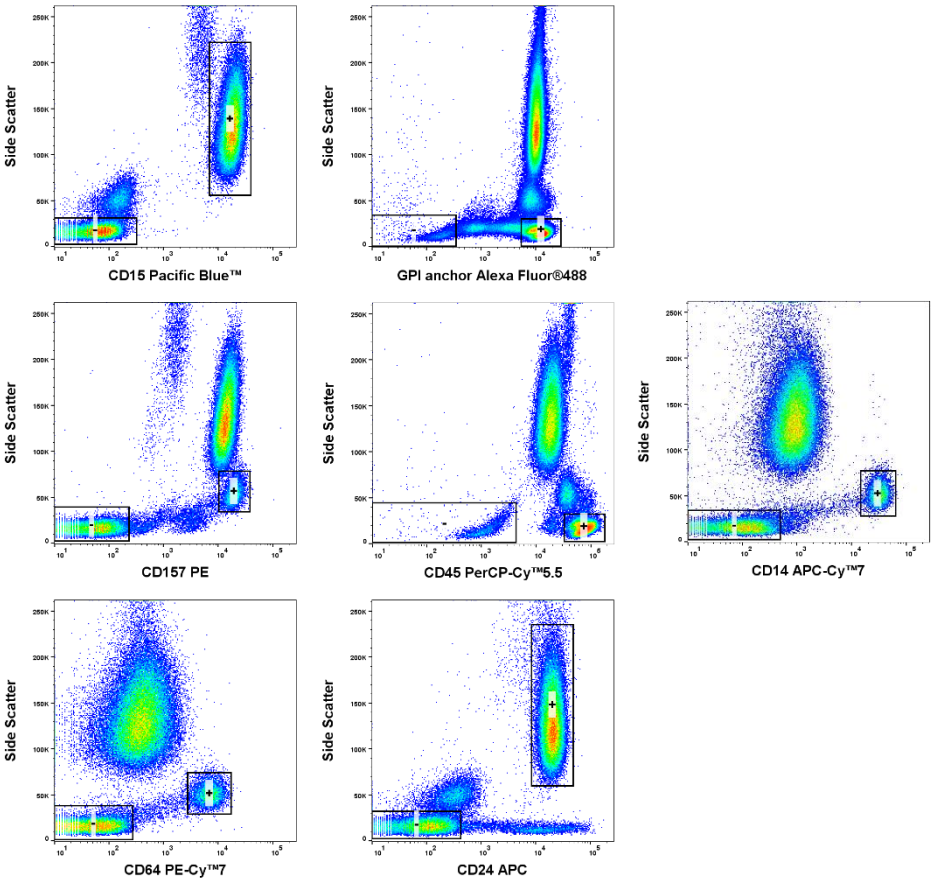
Figure 1 Identification of positive (+) and negative (-) cytometry compensation particles in compensation tubes.



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.



PNH RBC 3-color tube (Red strip)

Due to low iRBC count in diluted blood specimen, acquire a minimum of 500.000 erythrocyte events for analysis. Acquisition of ≥ 500.000 events results in long acquisition times. This may affect the 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 Check all acquired events in a dot-plot, Time vs. CD235a FITC.

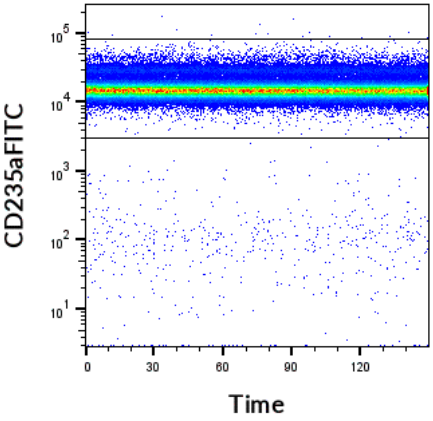
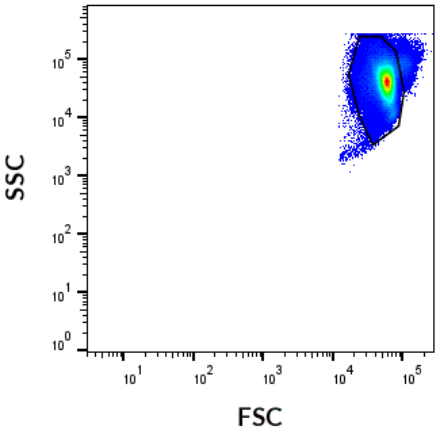
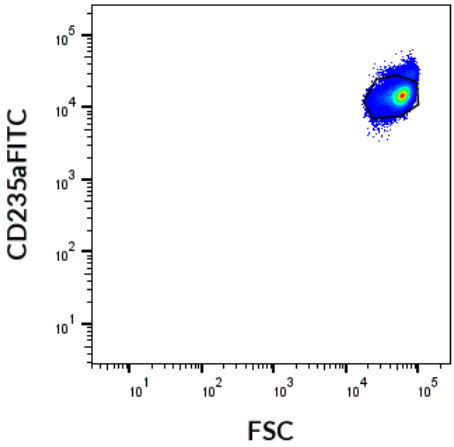


Figure 3a Display all events from the Time gate in a dot-plot FSC-A vs. SSC-A in logarithmic mode.



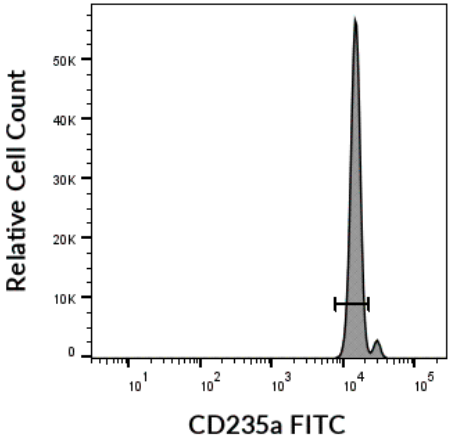
Visualize compensated data from the SSC vs. FSC gate in a dot-plot in logarithmic mode, where the X-axis represents FSC and the Y-axis represents fluorescence intensity in the FITC channel. Set “CD235a+ RBC singlets” gate (Figure 4).

Figure 4 Delineation of CD235a+ RBC singlets.



Optional:
For better differentiation of singlets is possible to visualize compensated data as a histogram where the X-axis represents fluorescence intensity in the FITC channel. Set “CD235a+ RBC singlets” gate (Figure 4a).

Figure 4a Delineation of CD235a+ RBC singlets.



Erythrocytes

Visualize CD235a+ RBC singlets in a dot-plot CD59 PE versus CD235a FITC. Separate events into three populations using three appropriate gates (Figure 5a, 5b) and calculate the percentage of events in the regions of Type I, Type II and Type III. For better adjustment of regions between Type II and III in some situations, a histogram can be useful.

Figure 5a Patient with PNH clone: A) CD235a+ RBC singlets in a dot-plot CD59 PE vs. CD235a FITC; B) CD235a+ RBC singlets in a CD59 PE vs. histogram.

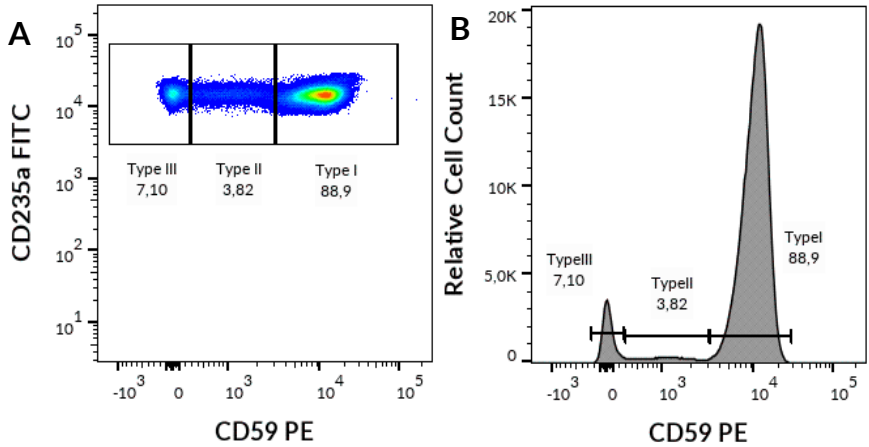
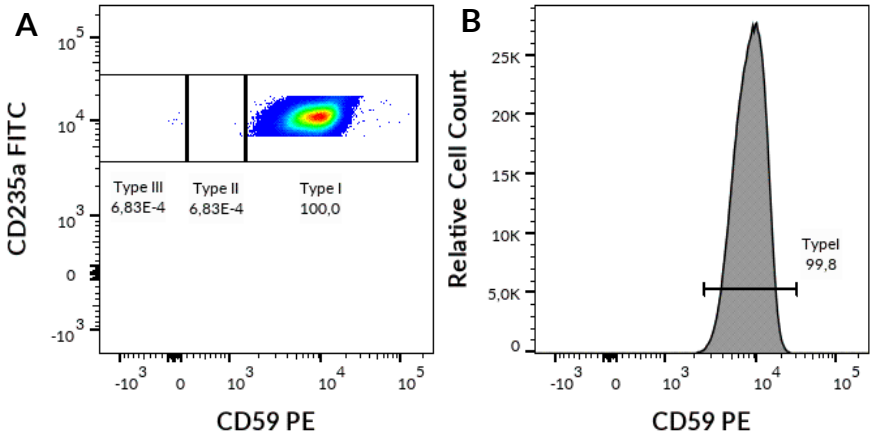


Figure 5b Healthy donor: A) CD235a+ RBC singlets in a dot-plot CD59 PE vs. CD235a FITC; B) CD235a+ RBC singlets in a CD59 PE vs. histogram.

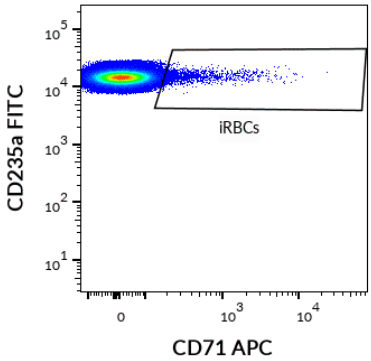


iRBCs (immature reticulocytes)

In patients receiving blood transfusions, it may be difficult to determine the true percentage of PNH type II and III RBCs. Healthy RBCs from a blood donor circulating in PNH patient`s bloodstream impact accurate PNH clone size percentage. The addition of CD71 may be helpful to gate out the donor's transfused cells [(2)].

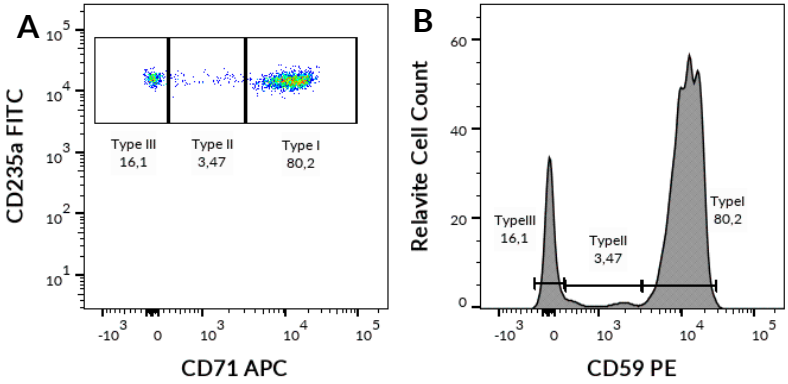
Visualize CD235a+ RBC singlets in a dot-plot CD71 APC versus CD235a FITC and separate CD71+ iRBCs (Figure 6).

Figure 6 CD235a+ RBC singlets in a dot-plot CD71 APC vs. CD235a FITC. Delineation of CD71+ iRBCs.



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

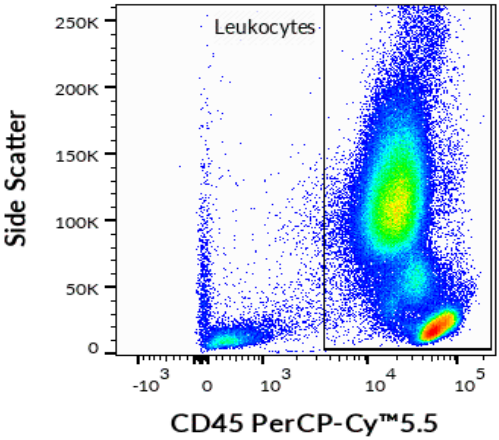
Figure 7 Patient with PNH clone, but without transfusion: A) CD71+ iRBCs in a dot-plot CD59 PE vs. CD235a FITC; B) CD71+ iRBCs in a CD59 PE vs. histogram.



PNH WBC 7-color tube (Cyan strip)

Acquire at least 50,000 neutrophils and 10,000 monocytes, which means at least 200.000 events for analysis are acquired. Visualize compensated data in a dot-plot side-scatter versus fluorescence intensity in PerCP-Cy™ 5.5. Set CD45+ leukocytes gate as shown in Figure 8.

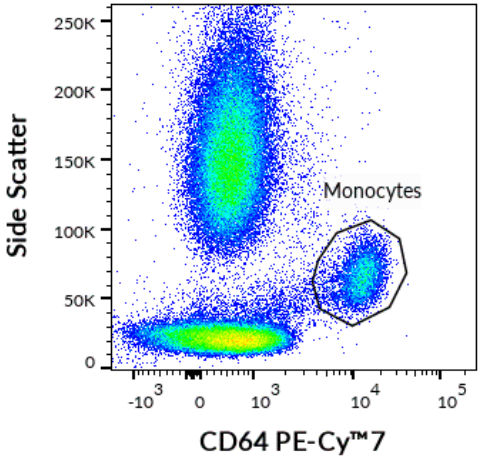
Figure 8 Delineation of CD45+ Leukocytes.



Monocytes

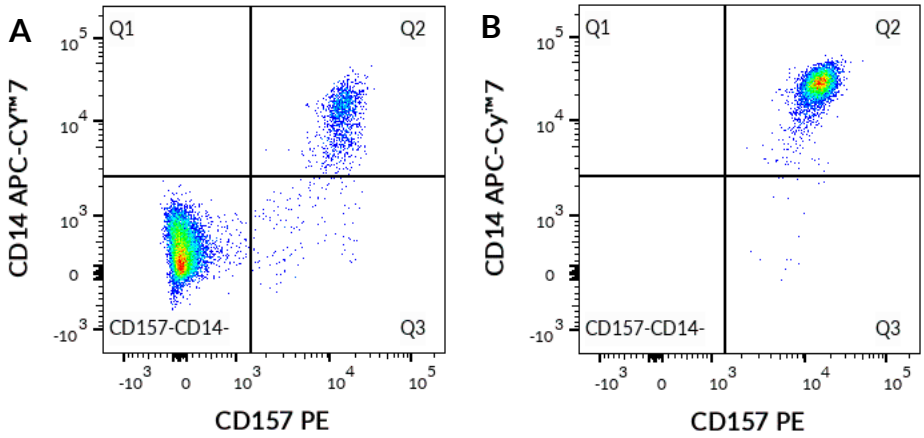
Visualize CD45+ leukocytes in a dot-plot side-scatter versus CD64 PE-Cy™7 and delimit CD64+ monocytes as shown in Figure 9.

Figure 9 Delineation of CD64+ Monocytes from Leukocytes.



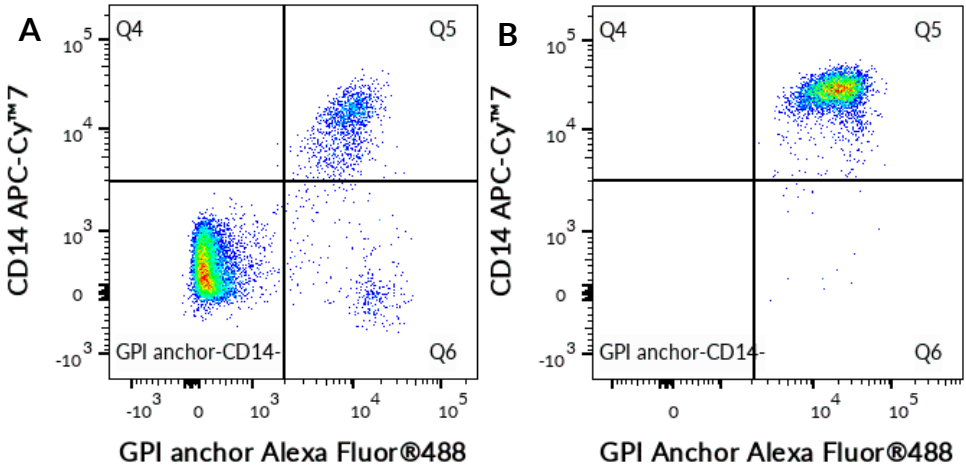
Visualize CD64+ monocytes in a dot-plot CD157 PE versus CD14 APC-Cy™7 (Figure 10). Set appropriate gates and calculate the percentage of CD157-CD14- population.

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



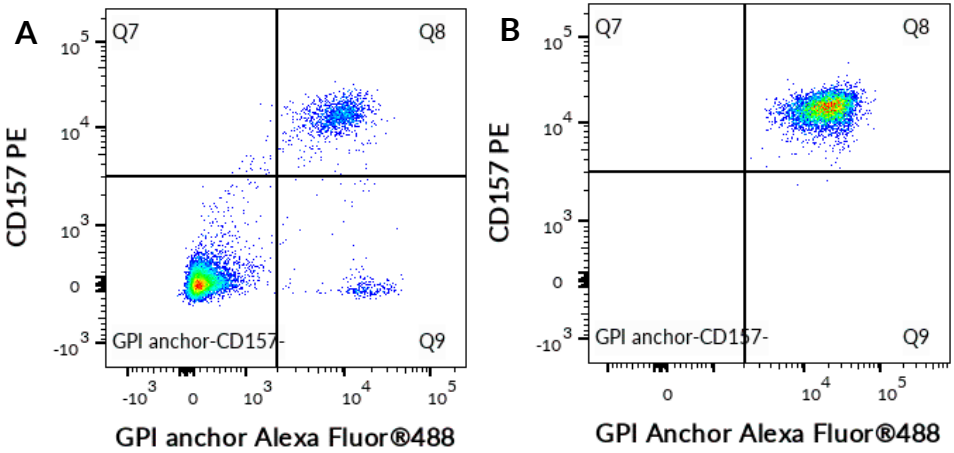
Then visualize the same CD64+ monocytes in a dot-plot GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD14 APC-Cy™7 (Figure 11). Set appropriate gates and calculate the percentage of GPI anchor-CD14- population.

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



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

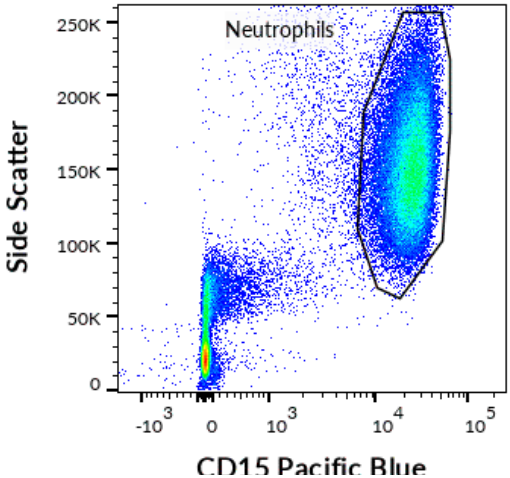
Figure 12 CD64+ Monocytes in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD157 PE. A) patient with PNH clone; B) healthy donor



Neutrophils

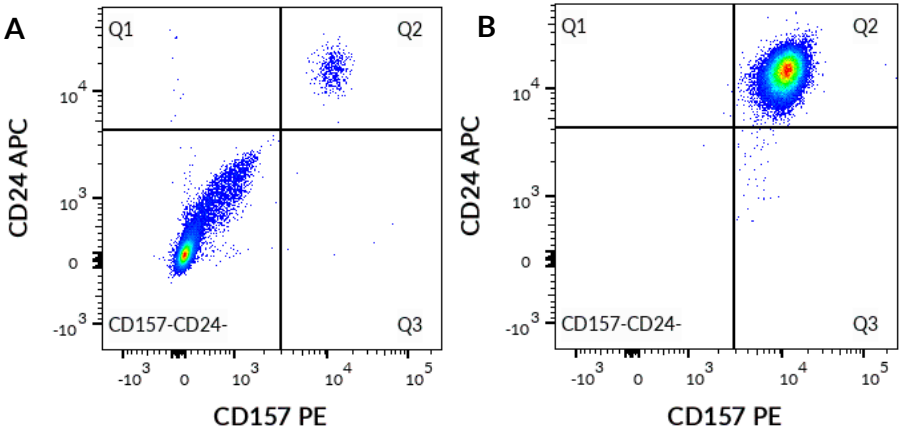
Visualize CD45+ leukocytes in a dot-plot side-scatter versus CD15 Pacific Blue™ and separate CD15+ neutrophils as shown in Figure 13.

Figure 13 Delineation of CD15+ Neutrophils from Leukocytes.



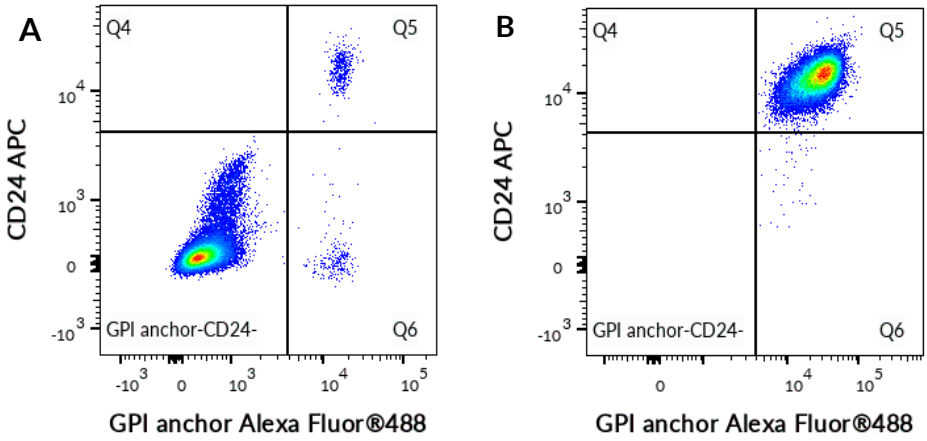
Visualize CD15+ neutrophils in a dot-plot CD157 PE versus CD24 APC as shown in Figure 14. Set appropriate gates and calculate the percentage of CD157-CD24- population.

Figure 14 CD15+ Neutrophils in a dot-plot CD157 PE vs. CD24 APC.
A) patient with PNH clone; B) healthy donor



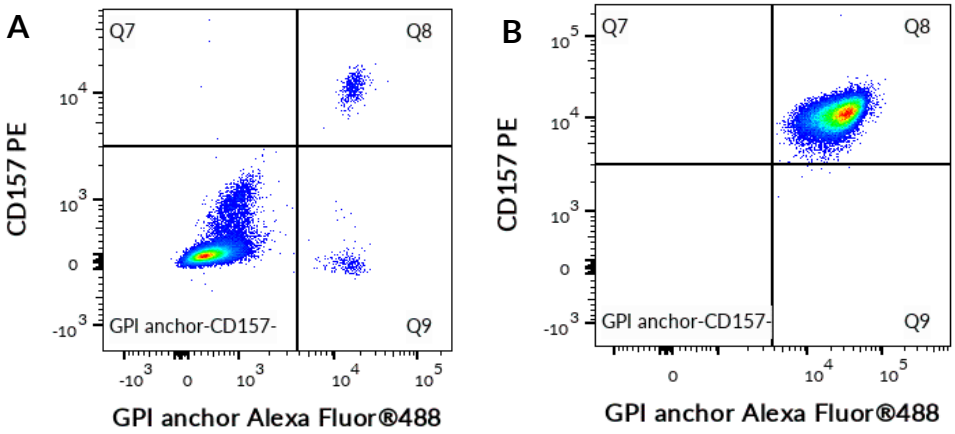
Then visualize the same CD15+ neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD24 APC. Set appropriate gates and calculate the percentage of GPI anchor-CD24- population as shown in Figure 15.

Figure 15 CD15+ Neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD24 APC.
A) patient with PNH clone; B) healthy donor



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

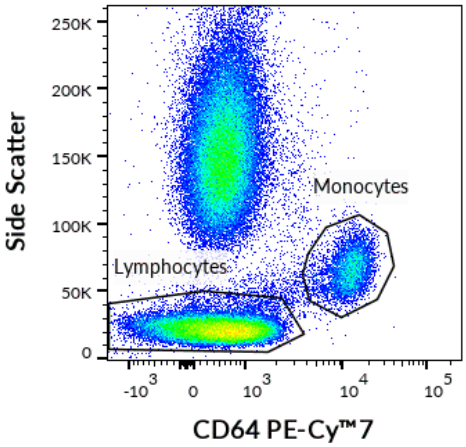
Figure 16 CD15+ Neutrophils in a dot-plot GPI anchor Alexa Fluor® 488 vs. CD157 PE. A) patient with PNH clone; B) healthy donor



Internal control

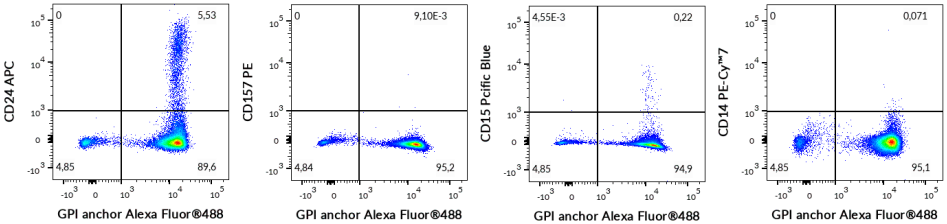
In order to verify fluorescence detector voltage and compensation, it is necessary to check the internal control (Figure 18). In the CD64 PE-Cy™7 versus side scatter plot, gate lymphocytes as the population for internal control (Figure 17).

Figure 17 Delineation of CD64- Lymphocytes from Leukocytes.



Then visualize lymphocytes in dot-plots GPI anchor Alexa Fluor® 488 (Proaerolysin) versus CD24 APC/CD157 PE/CD15 Pacific Blue™/CD14 PE-Cy™7 for verification of voltage settings, compensation, and antibody performance (Figure 18).

Figure 18 Internal controls.



References

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2) Illingworth, A et al. ICCS/ESCCA Consensus Guidelines to detect GPI-deficient cells in Paroxysmal Nocturnal Hemoglobinuria (PNH) and related Disorders Part 3 – Data Analysis, Reporting and Case Studies. Cytometry Part B 2018; 94B: 49– 66.

Trademarks

Revision History

Version 4, ED7787_TDS_v4

1) Chapter „Specimen“ was updated.

2) Cautions in Chapter “Procedure” were updated.

3) Sections „PNH RBC 3-color tube“ and „PNH WBC 7-color tube,“ including figures in chapter “Procedure”, were updated.

4) Section „Internal Controls“ in chapter “Procedure” was added.

7) Section „References“ article numbering has been corrected.

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