SpermFlowEx Kit

25 tests | Cat. No. **ED7079**

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7079_TDS_v7_EN

Date of Issue: 21-12-2024

Symbols used in the product labeling

RUO	Research Use Only		Keep Dry Keep away from rain
	Manufacturer	CONTENTS	Contents
	Consult instructions for use		
	Contains sufficient for <n> tests		
REF	Catalogue number		
LOT	Batch code		
	Use by date		
	Temperature limit		
	Keep away from sunlight		

Description

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

The SpermFlowEx Kit is designed for the analysis of human semen using flow cytometry to determine:

- Sperm count
- Leukocyte count
- Sperm viability
- Sperm acrosome integrity
- Presence of intra-acrosomal protein in sperm

Sperm count and leukocyte count

The measurement of sperm count and leukocyte count involves adding an internal standard (fluorescent beads with a known concentration) to the semen sample. Leukocytes are detected using a fluorescently labeled antibody against the human CD45 antigen (CD45 PE-Cy5).

Sperm viability

Sperm viability is determined using propidium iodide dye, which enters cells with damaged membranes (dead cells) and binds to their DNA.

Acrosome integrity

Acrosome integrity is assessed by detecting an intra-acrosomal protein (IAP), which is located inside the sperm acrosome. If the acrosome is intact, IAP remains undetectable. However, when the sperm membrane is damaged, IAP is exposed and becomes accessible to an antibody against IAP, allowing for detection of the protein.

Presence of intra-acrosomal protein

After permeabilizing the sperm membrane, intra-acrosomal protein (IAP) is detected using an antibody specific to IAP. If the sperm cell lacks IAP, it will not be detected after permeabilization.

The analysis should be complemented by the examination of sperm motility and morphology using a light microscope, in accordance to WHO recommendation[1].

Infertility has become a growing issue in the human population, with nearly 20% of couples affected, one-third of which is attributed to male factors. Therefore, semen analysis plays a crucial role in non-invasive diagnostic procedures that can help confirm or exclude an andrological cause of infertility.

Sperm examination is typically performed using the light microscope, following the WHO criteria set in 2010[1]. Such examination is subjective and depends on the

personal experience of the examiner. In contrast, flow cytometry offers a more balanced and objective analysis, as it measures a larger number of sperm cells and detects cells using specific staining[2].

Key parameters that significantly impact sperm's ability to fertilize include: sperm count, sperm viability, acrosome integrity, and the presence of an intra-acrosomal protein (IAP)[3]. The acrosome contains digestive enzymes, such as hyaluronidase and acrosin, which break down the outer membrane of the ovum called the zona pellucida, enabling the sperm's haploid nucleus to penetrate into the ovum. Addditonaly, the presence of leukocytes in semen indicates inflammation or a potential venereal disease.

Reagent(s) provided

Contents

The product SpermFlowEx Kit is sufficient for 25 tests and is provided with the following reagents:

Intra-acrosomal protein FITC ED7079-1 (1 vial) containing 0.5 ml of fluorescein-labeled mouse monoclonal antibody against intra-acrosomal protein (clone Hs-8).

CD45 PE-Cy5 ED7079-2 (1 vial) containing 0.25 ml of PE-Cy™5 labeled mouse monoclonal antibody against CD45 (clone MEM-28).

Fluorescent Count Standard ED7079-3 (1 vial) containing 2 ml of fluorescent beads, 1×10^6 particles/ml.

Propidium Iodide ED7079-4 (1 vial) containing 0.25 ml of propidium iodide solution**.**

Permeabilizing Solution ED7079-5 (1 bottle) containing 25 ml of permeabilizing reagent.

Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

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

Equipment required

Automatic pipette with disposable tips (10 µl – 1 ml) for pipetting specimen and reagents

Vortex mixer

Centrifuge

Light microscope

Flow cytometer with laser excitation source (488 nm), detectors for scattered light,

optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 2.

Table 2 Spectral characteristic of fluorochromes use in the product

Flurochrome	Excitation [nm]	Emission [nm]
FITC	488	525
PE-Cy™5	488	670
Propidium iodide	535	617

Storage and handling

Store at 2-8 °C.

Avoid prolonged exposure to light.

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

GHS Hazard Classification

WARNING: **Permeabilizing Solution** (ED7079-5) contains ethanol (CAS No. 64-17-5) in concentrations classified as hazardous.

Label elements	**Signal word**
	**Warning**
H-phrases	H226: Flammable liquid and vapour.
P-phrases	P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P260: Do not breathe spray. 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. P403+P235: Store in a well-ventilated place. Keep cool. P501: Dispose of contents/container to in accordance with local regulations.

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 Intra-acrosomal protein FITC reagent is a yellow-green liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of the CD45 PE-Cy5 reagent is a deep red to magenta liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of the Propidium Iodide is a pinkish-red liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of the Fluorescent Count Standard is a clear liquid. Since it is supplied in light-impermeable black vials, the appearance cannot be assessed by the user, but do not use the reagent if any change in appearance, for example turbidity or signs of precipitation, are suspected upon opening.

Normal appearance of the Permeabilizing Solution is a clear liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Limitation of use

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

Specimen

Semen samples should be measured within 8 hours after collection. Store at room temperature.

Check the sperm count using a light microscope. Dilute the semen 1:1 or 1:9 with PBS according to the sperm count (the greater the count the greater the dilution).

In the case of low sperm count (less than 10×10^6 /ml) the results of sperm count analysis will be affected by debris present inside the sperm cell region.

Procedure

Preparation of reagent(s) provided

Allow the Permeabilizing Solution to equilibrate to room temperature (20-25 °C) before use.

Sperm and leukocyte count

1. Pipette 50 µl of diluted semen into the bottom of the test tubes.
2. Add 10 µl of CD45 PE-Cy5.

   Vortex to mix and incubate for 20 minutes at room temperature.
3. Add 50 µl of Fluorescent Count Standard (first, mix the standard well and put a few drops into a clean microtube, then pipette the exact volume of the standard from the microtube to the sperm sample tube).
4. Add 0.5 ml of PBS, mix and analyze using a flow cytometer.

Sperm viability

1. Pipette 50 µl of diluted semen into the bottom of the test tubes.
2. Add 10 µl of Propidium Iodide solution.
3. Mix and incubate for 20 minutes at room temperature.
4. Add 0.5 ml of PBS, mix and analyze using a flow cytometer.

Acrosome integrity

1. Pipette 50 µl of diluted semen into the bottom of the test tubes.
2. Add 1 ml of PBS, mix and centrifuge for 5 minutes at 150× g. Remove supernatant.
3. Wash one more time (repeat step 2).
4. Add 10 µl of Intra-acrosomal protein FITC antibody to the pellet.
5. Mix and incubate for 20 minutes at room temperature.
6. Add 0.5 ml of PBS, mix and analyze using a flow cytometer.

Presence of intra-acrosomal protein

1. Pipette 50 µl of diluted semen into the bottom of the test tubes.
2. Add 1 ml of Permeabilizing Solution which was allowed to warm up to room temperature (20-25 °C).
3. Mix and incubate for 15 minutes at room temperature.

4. Centrifuge for 5 minutes at 150× g. Remove supernatant.
5. Add 1 ml of PBS to the pellet, mix and centrifuge for 5 minutes at 150× g.
6. Wash one more time (repeat step 5).
7. Add 10 µl of Intra-acrosomal protein FITC antibody to the pellet.
8. Mix well and incubate for 20 minutes at room temperature.
9. Add 0.5 ml of PBS, mix well and analyze using a flow cytometer.

Flow cytometry analysis

The flow cytometer selected for use with the product SpermFlowEx 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 Equipment required.

Set voltages on the 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.

Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

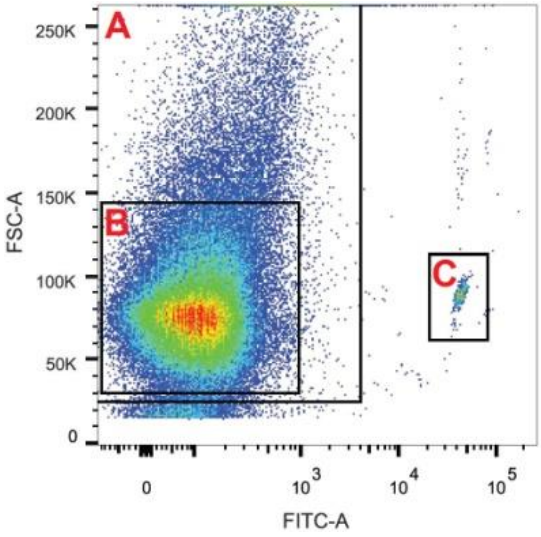
Fluorescent Count Standard is detected in FITC fluorescence detector as well as the monoclonal antibody against intra-acrosomal protein. Fluorescence of propidium iodide and of the PE-Cy™5 label is detected in PC5 or PerCP fluorescence detector.

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™).

Data analysis - Sperm and leukocyte count

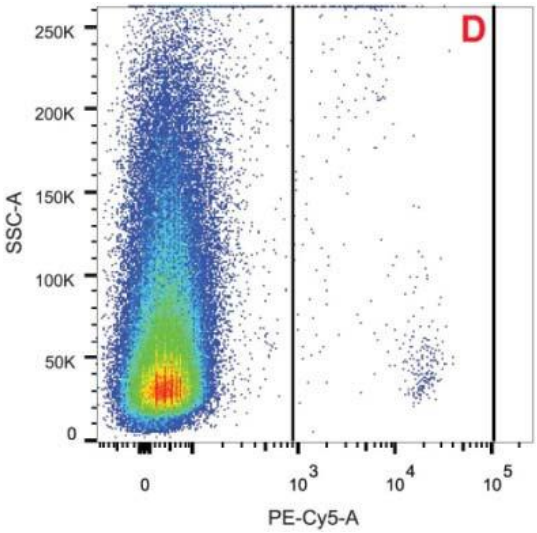
Visualize data as a dot-plot, where FITC fluorescence intensity is on the X-axis and forward-scatter (FSC) is on the Y-axis (Fig. 1). Set gates as shown in figure 1: gate **A** for all cells, gate **B** for spermatozoa and gate **C** for Fluorescent Count Standard (fluorescent beads).

Figure 1 Measurement of sperm count in semen.



Then visualize events from the gate A as a dot-plot, with the X-axis representing fluorescence intensity of CD45 PE-Cy5 staining and the Y–axis representing side-scatter (SSC) (Fig. 2). In contrats to to spermatozoa, leukocytes are CD45 positive. Separate the leukocytes by selecting the CD45 PE-Cy5 bright population with gate **D,** as shown in figure 2.

Figure 2 Measurement of leukocyte count in semen.



Calculate sperm and leukocyte count using formulas:

$S = \frac{gateB}{gateC} x\ dilution$ [10^6 sperm/ml]

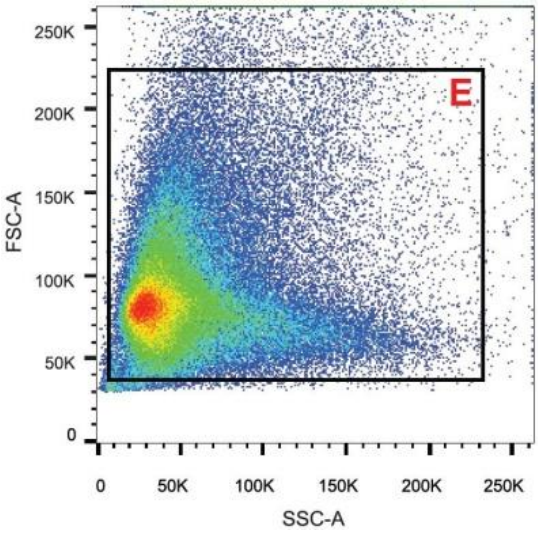
$L = \frac{gateD}{gateC} x\ dilution$ [10^6 leukocytes/ml]

S represents sperm count in the sample
L represents leukocyte count in the sample
gate B represents event count in gate B
gate C represents event count in gate C
gate D represents event count in gate D

Data analysis – Sperm viability

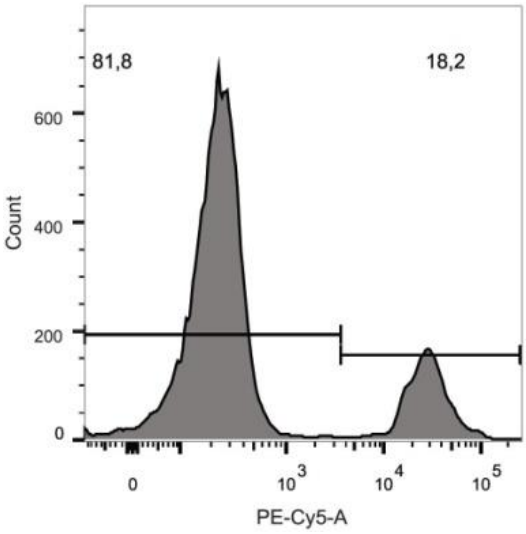
Visualize data as a forward-scatter (FSC) versus side-scatter (SSC) dot-plot. Set the gate around sperm cells as shown in figure 3.

Figure 3 Spermatozoa gating.



Next, visualize sperm cells in a histogram, where the X-axis represents fluorescence intensity of propidium iodide (Fig. 4). Separate positive and negative sperm cells using appropriate gate. Unstained (negative) population represents viable sperms, while positive population represents non-viable sperms. Sperm viability is indicated by the percentage of viable sperm cells relative to the total sperm population.

Figure 4 Measurement of sperm viability.



Data analysis – Acrosome integrity and presence of intra-acrosomal protein

Visualize antibody stained (intra-acrosomal protein FITC) permeabilized and non-permeabilized samples as the forward-scatter (FSC) versus side-scatter (SSC) dot-plot and set a gate E around sperm events (Figure 3). Next, visualize the gated events in histogram, where the X-axis represents fluorescence intensity in FITC fluorescence detector. Separate positive and negative sperms using appropriate gates (exmaples in Figure 5, 6, 7).

Figure 5 Non-permeabilized non-pathological sperms events with low content (< 30 %) of positive sperms. Negative events represent sperms with intact acrosome membrane and positive events represent sperms with damaged acrosome membrane.

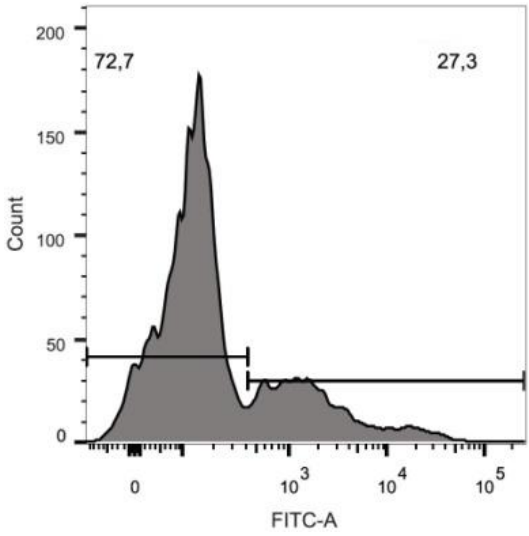


Figure 6 Permeabilized, non-pathological sperms with dominant content (100%) of positive sperm events. Positive sperm events indicate the presence of the intra-acrosomal protein.

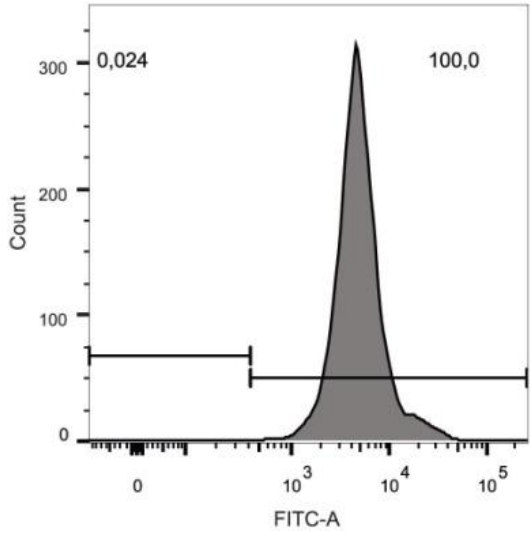
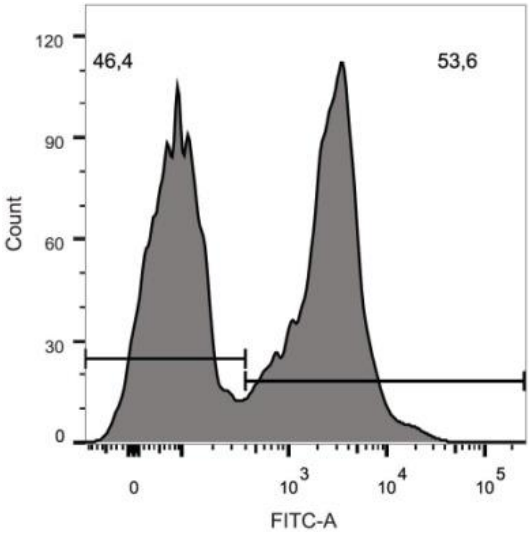


Figure 7 Non-permeabilized pathological sperms with high content (> 30 %) of positive sperm events with damaged acrosome membrane.



Expected values

Normal values according to WHO[1]

Sperm count in semen	> 15×10^6 /ml
Leukocyte count in semen	< 1×10^6 /ml
Sperm viability	> 58 %

Normal acrosomal values

Acrosome integrity of sperms (=percentage of positive sperm cells in non-permeabilized sample)	<30%
Presence of intra-acrosomal protein (=percentage of positive sperm cells in permeabilized sample)	> 90%

References

1) WHO laboratory manual for the Examination and processing of human semen. World Health Organization, 5th edition, 2010.
2) Gilan L, Evans G, Maxwell WM (2005) Flow cytometric evaluation of sperm parameters in relation to fertility potential. Theriogendogy. 63: 445-57.
3) Peknicova J, Chladek D, Hozak P (2005) Monoclonal antibodies against sperm intra-acrosomal antigens as markers for male infertility diagnostics and estimation of spermatogenesis. Am J Reprod Immunol. 53(1): 42-9.

Use of Third Party Trademarks

Revision History

Version 7, ED7079_TDS_v7

The layout design has been updated, and the text contains minor revisions for improved readability.

Manufacturer

EXBIO Praha, a.s.
Nad Safinou II 341
25250 Vestec
Czech Republic

Contact Information

info@exbio.cz
technical@exbio.cz
orders@exbio.cz
www.exbio.cz

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