SpermFlowEx Kit

Cat.No. ED7079

Description

The SpermFlowEx Kit is designed for analysis of following parameters in human semen using flow cytometry:

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

Sperm count and leukocyte count

Measurement of the sperm count and leukocyte count is based on the addition of an internal standard (fluorescent beads with known concentration) to the semen sample. Detection of leukocytes in the sample is performed by staining with labeled antibody against human CD45 antigen (CD45 PE-Cy5).

Sperm viability

Sperm viability is examined using propidium iodide which permeates through damaged membranes of dead cells and binds to their DNA.

Acrosome integrity

Measurement of acrosome integrity is based on the detection of an intra-acrosomal protein (IAP), which can be found inside the acrosome. If the sperm acrosome is intact, it is unable to detect IAP. Sperm with damaged membrane has IAP exposed and therefore accessible to the antibody against IAP, hence the protein is detected.

Presence of intra-acrosomal protein

After permeabilization of sperm membrane, intra-acrosomal protein is exposed to the antibody against intra-acrosomal protein (IAP) and thus is detected. In case, that sperm does not contain intra-acrosomal protein (IAP), the protein is not detected after permeabilization.

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

Lately, infertility of human population is a growing problem. Nearly 20 % of couples suffer from infertility, which is in 1/3 causes attributed to a male factor. Therefore, analysis of semen should play a role in basic non-invasive examination, which could verify or negate andrological cause of infertility.

Sperm examination is carried out mainly using the light microscope and obeys the WHO criteria from 2010[1]. Such examination is subjective and depends on the personal experience of the examiner. When using the flow cytometry the analysis is balanced and objective, since the amount of measured sperms is much greater and detection of cells is provided via specific staining[2]. Main parameters having great impact on the ability of sperm to fertilize are: sperm count, sperm viability, acrosome integrity and presence of an intra-acrosomal protein (IAP)[3]. The acrosome contains digestive enzymes (including hyaluronidase and acrosin). These enzymes breakdown the outer membrane of the ovum called the zona pellucida, allowing the haploid nucleus of the sperm penetrate into the ovum. Presence of leukocytes in semen is a mark of an actual inflammation or a venereal disease.

Specification

Intra-acrosomal protein FITC contains mouse monoclonal antibody against intra-acrosomal protein, FITC labeled (Ready to use).

CD45 PE-Cy5 contains mouse monoclonal antibody against CD45, PE-Cy™5 labeled (Ready to use).

Fluorescent Count Standard contains fluorescent beads, 1×10^6 particles/ml (Ready to use).

Propidium Iodide contains propidium iodide solution (Ready to use).

Permeabilizing Solution contains permeabilizing reagent (Ready to use).

Reagents provided

ED7079-1 **Intra-acrosomal protein FITC**, 0.5 ml
ED7079-2 **CD45 PE-Cy5**, 0.25 ml
ED7079-3 **Fluorescent Count Standard**, 2.0 ml
ED7079-4 **Propidium Iodide**, 0.25 ml
ED7079-5 **Permeabilizing Solution**, 25 ml
The content of the kit is sufficient for 25 tests.

Materials required but not provided

5ml test tubes (12 × 75 mm)
Deionized water (dH_2O)
Phosphate buffered saline (PBS)

Storage and handling

Store the SpermFlowEx Kit at 2-8 °C. Expiration date is printed on reagent labels and on the kit outer packaging label.

Warnings and precautions

- Intended for research use only.
- Do not use reagents after the expiration date.
- Avoid contamination of reagents.
- Avoid prolonged exposure of the reagents to light.
- **Permeabilizing Solution** contains ethanol (<50%).
  The solution is classified as hazardous according the Regulation (EC) No 1272/2008.
  H phrases
  H226 Flammable liquid and vapours.
  P phrases
  P210 Keep away from heat/sparks/open flames/hot surfaces. – No smoking.
  P260 Do not breathe vapours/spray.
  P280 Wear protective gloves / eye protection / face protection.
  P301+P312 IF SWALLOWED: Call a POISON Center or doctor/physician if you feel unwell
  P302+P352 IF ON SKIN: Wash with plenty of soap and water.
  P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  P501 Dispose of contents/container to authorized facility for dangerous wastes.
  See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product.
- Semen samples are considered as potentially infectious and must be handled with care.
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure the stable sensitivity of detectors.
- Concentration of reagents was optimized to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test.
- The monoclonal antibody against Intra-acrosomal protein cannot be used for specific identification of human sperms due to the antibody binding to cell debris or potential cross reactivity with other isoforms of target antigen (sperm-specific glyceraldehyde phosphate dehydrogenase) present in other cell types.
- In case of reagents deterioration or if data obtained show any performance alteration, please contact manufacturer using following e-mail address: technical@exbio.cz

Application

Human semen analysis

Specimen

Use semen samples within 8 hours after ejaculation. Store semen samples 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 (less than 10 x 10^6 /ml) sperm count, we recommend to check the sperm count using microscope. In such samples the results might be affected due to relatively higher content of cell debris in the gated region.

Required for handling

Automatic pipettes with disposable tips
Vortex mixer
Centrifuge with rotor suitable for 5ml tubes
Light microscope
Flow cytometer - blue laser excitation 488 nm and proper filters

Procedure

Sperm and leukocyte count

1. Pipette 50 µl of diluted semen to a tube.
2. Add 10 µl of **CD45 PE-Cy5** reagent.
3. Mix the sample and incubate for 20 minutes at room temperature.
4. 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).
5. Add 0.5 ml of PBS, mix and analyze using a flow cytometer.

Sperm viability

1. Pipette 50 µl of diluted semen to a tube.
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 to a tube.
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 to a tube.
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 Cytometric Analysis

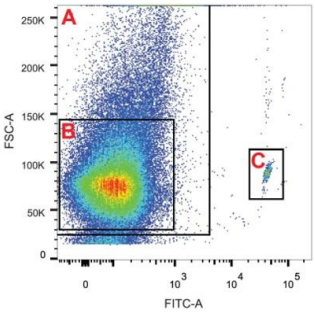
Analyze samples using flow cytometer equipped with a 488 nm excitation laser and appropriate filter set-up.

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.

Sperm and leukocyte count

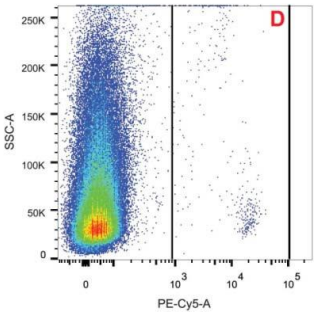
Visualize measured data as a dot-plot, where FITC fluorescence intensity is on the X-axis and forward-scatter (FSC) is on the Y-axis (Fig. 1). Set gates as shown in figure 1: gate **A** for all cells, gate **B** for sperms and gate **C** for Fluorescent Count Standard (fluorescent beads).

Figure 1 Measurement of sperm count in semen



Then visualize events from the gate A as a dot-plot (Fig. 2), where the X-axis represents fluorescence intensity of PE-Cy™5 dye and the Y–axis represents side-scatter (SSC). On the contrary to the sperms, leukocytes are CD45 positive. Separate the bright population of leukocytes using gate **D** as shown in figure 2.

Figure 2 Measurement of leukocyte count in semen



Calculate sperm and leukocyte count using following formulas:

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

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

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

Sperm viability

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

Then visualize sperm cells in a histogram (Fig. 4), where the X-axis represents fluorescence intensity of propidium iodide in PE-Cy5 or PerCP channel.

Separate positive and negative sperm cells using appropriate gate. Negative population represents viable sperms, while PI-positive population represents non-viable sperms. Viability of sperms is represented by the percentage of viable sperms from all sperm cells.

Figure 3 Sperm gating

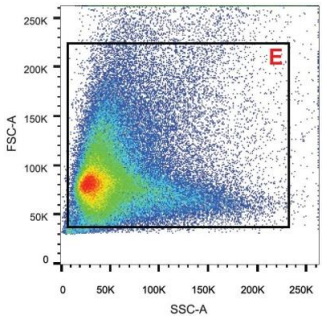
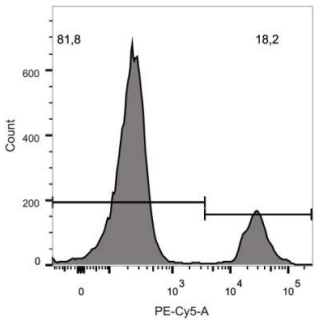


Figure 4 Measurement of sperm viability



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 around sperm events as shown in figure 3. Then visualize sperm events in a histogram (see Fig. 5-7), where the X-axis represents fluorescence intensity in FITC fluorescence detector. Separate positive and negative sperms using appropriate gates.

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

Figure 5 Non-permeabilized, non-pathological sperms

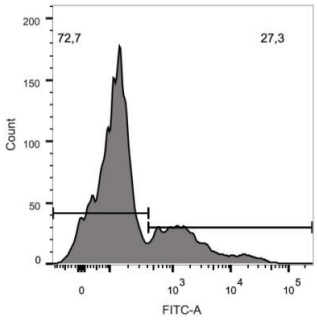


Figure 6 shows permeabilized non-pathological sperms with dominant content of positive sperm events. Positive sperm events indicate the presence of the intra-acrosomal protein.

Figure 6 Permeabilized, non-pathological sperms

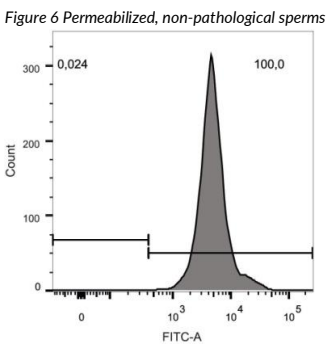
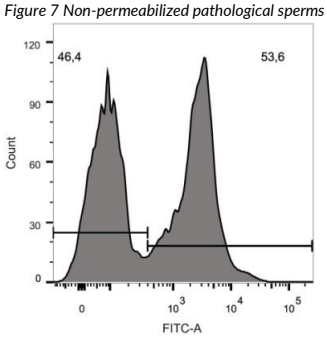


Figure 7 shows non-permeabilized pathological sperms with high content (> 30 %) of positive sperm events with damaged acrosome.

Figure 7 Non-permeabilized pathological sperms



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

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

Manufacturer

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Trademarks

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Revision History

- Version 1, ED7079_TDS_v1
Initial Release
- Version 2, ED7079_TDS_v2
Hazard and Precautionary statements updated.
- Version 3, ED7079_TDS_v3
Inserted a new limitation/recommendation with reagard to the samples of low sperm count.
- Version 4, ED7079_TDS_v4
Inserted a new limitation of use with regard to the specificity of the monoclonal antibody against Intra-acrosomal protein.
- Version 5, ED7079_TDS_v5
The company logo changed. TDS layout changed. Manufacturer postal code changed from 25242 to 25250.
- Version 6, ED7079_TDS_v6
In the Trademarks section was changed from GE Healthcare to Cytiva.

Symbols

REF	Catalog number
LOT	Batch code
	Use-by date
	Temperature limits
	Consult instructions for use
	Keep away from sunlight
	Manufacturer
RUO	For Research use only. Not for use in diagnostic or therapeutic procedures.

exbio

SpermFlowEx Kit

25 tests | Cat.No. ED7079

For Research use only.
Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet

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