

# LeucoScreen Plus

## Semi-quantitative histochemical kit for the determination of peroxidase-positive white blood cells in human semen

### LeucoScreen Plus

For *in vitro* diagnostic use only.  
Reagent for professional use only.

#### GENERAL INFORMATION

Most human ejaculates contain leucocytes and the predominant form of leucocytes in human semen are peroxidase-positive granulocytes<sup>1,2,3,4</sup>. Excessive presence of these cells (leucocytospermia) may indicate the existence of reproductive tract infection. Leucocytospermia may also be associated with defects in the semen profile (reduction in sperm motility and DNA integrity, raise of sperm viscosity as well as loss of sperm function as a result of oxidative stress, and/or secretion of cytotoxic cytokines by these white blood cells)<sup>5,6</sup>. Although leucocytospermia is not an absolute indication of infertility, this condition is observed on average in 10 to 20% of all infertile men<sup>7</sup>.

According to the World Health Organization (WHO), the presence of more than one million peroxidase positive white blood cells (WBC) per ml ejaculate is considered abnormal and is labelled as "leucocytospermia"<sup>4</sup>. However, this threshold is under debate, as some have found this value too low and others too high. Indeed, threshold levels from  $0.2 \times 10^6$  –  $2 \times 10^6$  have been reported<sup>7,9</sup>.

When the threshold of one million peroxidase positive WBC per ml ejaculate is exceeded, microbiologic tests should be performed to investigate if there is an accessory gland infection. Assessment of accessory gland markers can provide additional useful information about the proper functioning of the epididymis (EpiScreen Plus, FertiPro NV), seminal vesicles (Fructose Test, FertiPro NV) or prostate. Importantly, the absence of leucocytes does not exclude the possibility of an accessory gland infection.

#### INTENDED PURPOSE

LeucoScreen Plus is a semi-quantitative, non-automated, histochemical and diagnostic kit for the determination of peroxidase-positive white blood cells in human semen.

The number of tests that can be performed with the LeucoScreen Plus kit is not specified, instead, the kit has been designed for 40 days of analysis during the lifetime of the kit (20 work solutions can be made, which are stable for 2 consecutive days).

#### MATERIAL INCLUDED WITH THE TEST

Reagent 1 – 6 ml of Substrate solution (4-CN in methanol)  
Reagent 2 – 300 µl of 30% Hydrogen peroxide  
Reagent 3 – 22 ml of Buffer solution  
Reagent 4 – 1.2 ml of Counter stain solution

A certificate of analysis and MSDS are available on request or can be downloaded from our website ([www.fertipro.com](http://www.fertipro.com)).

#### MATERIAL REQUIRED, BUT NOT PROVIDED

Microscope slides, cover glasses, test tubes (Eppendorf), pipettes, bright field microscope.

**Note:** It is recommended to use a light microscope and not to use a phase-contrast microscope as the latter could lead to interpretation difficulties.

#### TEST PRINCIPLE

When a typical semen analysis is performed, it is very difficult to differentiate white blood cells from other types of round cells in the semen sample (for example spermatogenic progenitor cells<sup>10</sup>). LeucoScreen Plus makes use of the intrinsic peroxidase activity of certain white blood cells and can therefore be used to differentiate the peroxidase-positive white blood cells (e.g. polymorphonuclear (PMN) granulocytes and macrophages) in a human semen sample from other types of round cells.

In presence of Reagent 2 (H<sub>2</sub>O<sub>2</sub>), the myeloperoxidase in leucocyte granules will oxidize Reagent 1 (4-CN) to a bluish-purple precipitate (4-chloro-1-naphton). Reagent 4 contains a counter stain solution to facilitate the differentiation between peroxidase positive round cells and peroxidase negative round cells.

#### METHOD

Before first use of the LeucoScreen Plus kit, we strongly recommend to follow the training program and to view our demonstration video, which are both available on our website. The video can also be downloaded by scanning this QR-code:



#### Specimen types

The test should be performed on fresh human semen samples containing more than  $1 \times 10^6$  round cells per ml.

#### Specimen collection

Standard semen collection containers should be used, typically in polypropylene and sperm survival/sperm motility tested, when semen is collected by masturbation. Non-semen toxic plastic condoms should be used when semen collection by masturbation is discouraged. Keep the semen collection container at room temperature before adding the semen sample in order to avoid large changes in temperature that may affect spermatozoa. The test should be performed within the same day of semen collection.

#### Warning before use

Do not use the product if seal of the bottles is opened or defect when the kit is delivered.

#### Reagent preparation

Reagent 1 should contain a clear fluid, do not use the kit if the fluid has turned yellow.

#### Method

- Count the number of round cells whilst determining the sperm concentration during routine semen analysis. Calculate and write down the total concentration of round cells in mill/ml, as this will be needed for the calculation of the concentration peroxidase-positive white blood cells. When round cells concentration exceeds  $1 \times 10^6$  per ml, the LeucoScreen Plus test is indicated.
- Prepare working solution: Add the following volumes to an Eppendorf tube and mix thoroughly.
  - 200 µl Reagent 1
  - 5 µl Reagent 2
  - 1 ml Reagent 3
 This working solution is stable for 48 hours when stored between 2-8 °C, protected from (sun)light.
- Take 10 µl of the semen sample and add 20 µl of working solution. Mix thoroughly.
- Incubate for two minutes at room temperature **in the dark**.
- Transfer 10 µl of the mix to the middle of a microscope glass and add 10 µl of Reagent 4. Mix thoroughly using the edge of the cover slip.
- Cover with the cover slip.
- Count and classify a total of 200 round cells using a magnification of 400x. Scan different microscopic fields (preferably 20).
- Discard after each individual test, all used reagents and materials.

**Note:** In some semen samples, you might prefer to enrich the round cell concentration to facilitate cell counting. Therefore, centrifuge the sample for 15 minutes at 350g, remove some volume of the semen sample and resuspend the pellet. In case of very high concentration of round cells (i.e. above  $20 \times 10^6$  per ml), it is strongly advised to dilute the sample in PBS or in FertiCult™ Flushing medium.

#### INTERPRETATION

- Peroxidase positive round cells: contain (bluish-) black granules and have an anthracite-grey cytoplasm. This anthracite area can cover the complete cell or only part of it.
- Peroxidase negative round cells: remain unstained or can be pink-coloured. Granules seen in unstained cells are not surrounded by the typical anthracite color seen in positive cells.

#### CALCULATION OF THE CONCENTRATION OF PEROXIDASE-POSITIVE WHITE BLOOD CELLS

- Calculate the proportion of peroxidase-positive cells as follows:

PROPORTION POSITIVE ROUND CELLS	
Number of POSITIVE round cells	Number of POSITIVE round cells + Number of NEGATIVE round cells

- Now, calculate the concentration of peroxidase-positive white blood cells in the semen sample as follows:

CONCENTRATION (mill/ml)	
Proportion positive round cells	total concentration of round cells

Example:

- Total concentration of round cells is 2 mill/ml (determined during sperm concentration analysis)
- With the LeucoScreen Plus test, 120 round cells are found positive and 80 round cells are found negative
- Proportion positive round cells =  $120 / (120 + 80) = 0.6$
- Concentration of peroxidase-positive white blood cells =  $0.6 \times 2 \text{ mill/ml} = 1.2 \text{ mill/ml}$

#### LIMITATIONS OF THE METHOD

This test is an aid in the diagnosis of male infertility and, as for other biological tests, interpretation of the results must be performed within the framework of clinical findings and data of history taking. The LeucoScreen Plus test only stains peroxidase-positive WBC, other types of WBC (e.g. lymphocytes and monocytes) are not detected.

#### PERFORMANCE CHARACTERISTICS

Repeatability and reproducibility:  $CV_{intra} < 15\%$ ,  $CV_{inter} < 15\%$   
Percent positive agreement: 88%, percent negative agreement: 96%

#### STORAGE / DISPOSAL

- The kit is stable for at least 12 months after production date (even after opening).
- Do not use after expiry date mentioned on the product label.
- Store reagents between 2 °C and 25 °C.
- Do not freeze.
- Protect from (sun)light.
- The bottles must be kept tightly closed at all times.
- The working solution can be stored up to 48 hours at 2-8 °C, protected from (sun)light.
- The reagents need to be disposed in accordance with the local regulations for disposal of medical devices.



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#### MATERIAL INCLUDED

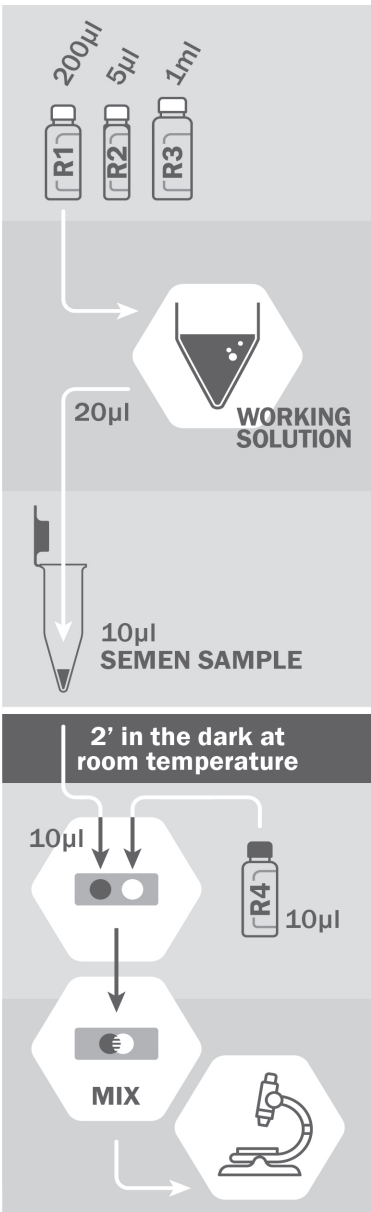
LEUCO\_PLUS LeucoScreen Plus - 40 days of analysis

#### CUSTOMER-TECHNICAL SUPPORT

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**Graphic presentation of the protocol:**



**PRECAUTIONS**

Use of gloves is advised.

*Reagent 1 contains methanol:* highly flammable liquid and vapour, toxic if swallowed, in contact with skin or inhaled, causes damage to organs.

*Reagent 2 contains H<sub>2</sub>O<sub>2</sub>:* harmful if swallowed or if inhaled; causes serious eye damage. Wear eye / face protection.

*Reagent 3 contains ProClin 950:* Causes severe skin burns and eye damage, may cause an allergic skin reaction.

All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always use fresh pipette tips for each step to avoid cross-contamination. Do not discard product into the environment.

Any serious incident (as defined in the European In Vitro Diagnostic Medical Device Regulation 2017/746) that has occurred should be reported to FertiPro NV and, if applicable, to the competent authority of the EU Member State in which the user and/or patient is established.

**BIBLIOGRAPHY**

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**SYMBOLS GLOSSARY**

Symbols as defined in ISO 15223

	Catalogue number
	Batch code
	Consult instructions for use
	Manufacturer
	In Vitro Diagnostics
	Temperature limit
	Use-by date
	Keep away from sunlight

Symbol as defined in IVDR 2017/746

	CE marking by Notified Body 2797
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Symbol as defined in regulation (EC) No. 1272/2008 [CLP]

	GHS08 Serious health hazard
	GHS02 Flammable liquid
	GHS06 Acute toxicity
	GHS07 Health hazard
	GHS05: Skin corrosion/irritation



Other languages can be downloaded on our website ([www.fertipro.com](http://www.fertipro.com))