

INSTRUCTIONS FOR USE

IVD

PCG® SPERM DNA FRAGMENTATION KIT

1. KIT CONTENT

REF	PI0100050	PI0100100
Package	50 tests	100 tests
Reagent A	1 x 6 mL	1 x 12 mL
Reagent B	1 x 12 mL	1 x 24 mL
Reagent C	1 x 34 mL	1 x 70 mL
Reagent D	1 x 250 µL	1 x 500 µL
Sperm preservation reagent	1 x 17 mL	1 x 33 mL

2. STORAGE AND TRANSPORTATION CONDITIONS

The kit will remain stable at least until the expiry date printed on the package if the storage temperature is kept at 2 – 8°C.

The mixture of Reagent C and Reagent D must be protected from light and can be used within 2 weeks when stored at 2 – 8°C and up to 1 year when stored at -20°C (within the shelf life of the test kit) after mixing.

3. TECHNICAL SPECIFICATIONS

Regulatory Status	IVD
Function	Screening
What is Detected/ Target	DFI (DNA fragmentation index)
Automated/ Manual detection	Manual
Type of Analysis	Quantitative
Validated Specimen	Semen sample
Intended User	Laboratory technician
Product Performance	- Reagent A, B, C are colorless, transparent liquids. - Reagent D is an orange liquid. - Sperm preservation reagent is a pale-yellow liquid. - Maximum value of the test: $DFI \leq 98\%$. - Repeatability: coefficient of variation (CV) $\leq 6\%$. - Reproducibility: coefficient of variation (CV) $\leq 10\%$



4. INDICATION AND METHOD PRINCIPLE

Indication	The test kit is recommended to be used to determine the degree of sperm DNA fragmentation in the following cases: • Couples with unexplained infertility • Recurrent miscarriages or early pregnancy loss • Couples with persistent infertility even after treatment of the female partner • Couples considering IUI, IVF, ICSI • Men over 40 years of age • Men who are smokers • Men on certain prescription medicines can affect sperm • Men who have been treated for cancer • Men exposed to toxic agents • Men who have had an Urinary Tract Infection (UTI) • Men considering varicocele repair
Principle	Detecting the vulnerability of sperm DNA to acid-induced DNA denaturation, followed by staining with the fluorescent dye acridine orange. Normal sperm chromatin remains double-stranded, while fragmented sperm chromatin would be converted into single-stranded after being denatured by acid. When excited at 488 nm, the complex of Acridine orange (AO), which is present in the mixture of Reagent C and Reagent D, and dsDNA exhibits green fluorescence (wavelength of 515 - 530 nm) while the complex of AO-ssDNA exhibits red fluorescence (wavelength of 630-650 nm). Sperm DNA fragmentation was assessed through the DFI (DNA fragmentation index).

5. USER MANUALS

SAMPLING AND SAMPLE STORAGE

- The semen specimen should be obtained by masturbation (collected in a specialized container) or sexual intercourse (using a special semen collection condom) after an abstinence period of 2-7 days, preferably 3-5 days.
- Fresh semen samples must be transported at a temperature of 37°C and processed within 5 hours and should be completely liquefied prior to testing. In case of longer storage, keep all samples in the Sperm Preservation Reagent at a 1:1 ratio at 2-8°C for up to 72 hours, -20°C for up to 7 days, and -80°C for up to 30 days.

For example: To store sperm, dispense 300 µL of semen into a tube containing 300 µL of Sperm Preservation Reagent. Mix thoroughly and gently with a pipette, then store at 2-8°C, -20°C, or -80°C.

- Frozen semen samples should be thawed by submerging in a 37°C water bath until the last trace of ice has melted and then placed at 37°C or room temperature.

REAGENT PREPARATION

- Reagent D is mixed with Reagent C at a ratio of 6 µL of Reagent D: 1 mL of Reagent C in a dark tube. Mix thoroughly.
- Calculate the total mixture volume of Reagent D and Reagent C for a batch by multiplying number of tests by 600 µL (the mixture volume each test).

For example: The total mixture volume for a batch of 50 samples will approximate 30 mL which is prepared by adding 180 µL of Reagent D to 30 mL of Reagent C.

- All reagents, buffers and samples must be kept on ice or cold gel (at 4-8°C) during handling.

SAMPLE PROCESSING

- Dilute the initial semen sample in Reagent A to a sperm concentration of $2-3 \times 10^6$ per mL with the total volume of diluted semen in a reaction is 100 μ L.

For example: Preparing 100 μ L of a sperm concentration of $2-3 \times 10^6$ per mL from an initial sperm concentration of $M \times 10^6$ per mL as below:

The volume of semen to be aspirated is $N = 2 \times 100 / M$ (μ L).

The volume of Reagent A is $(100-N)$ (μ L).

- Add 100 μ L of diluted semen to a processing 5 mL tube, 12x75 mm.
- Add 200 μ L of Reagent B to the processing tube, mixing gently.
- Incubate for 30 sec.
- Add 600 μ L of the mixture of Reagent C and Reagent D to the processing tube, mixing gently
- Place the processing tube into the flow cytometer and start run (not exceed 30 min).
- A population of desired cells at a rate of 100-300 cells per second. If the flow rate is exceeded 300 cells per second, repeat the run with the appropriate sample dilution.
- Data of 5,000 sperm cells are analyzed for each sample.

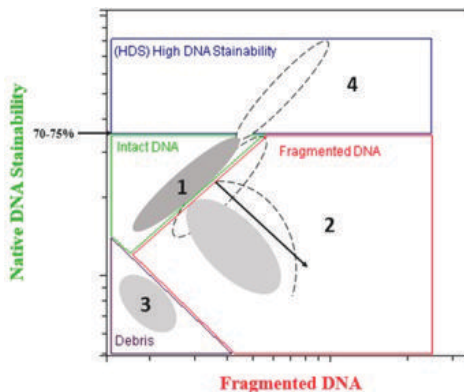
6. DATA ANALYSIS & RESULTS INTERPRETATION

- Noise signals and high DNA stainability (HDS) will be filtered and removed from data of 5,000 sperm cells
- The rate of DNA fragmentation or DNA fragmentation index (DFI) was determined by the following formular:

$$\text{DFI (\%)} = \frac{\text{Red fluorescence}}{\text{Total (red+green) fluorescence}} \times 100\%$$

Interpretation of results:

- ▶ Men with $\text{DFI} \leq 15\%$ (ideally $< 10\%$) is normal.
- ▶ Men with $15\% \leq \text{DFI} \leq 30\%$ is considered to have moderate level of DNA fragmentation and poor pregnancy outcomes.
- ▶ Men with $\text{DFI} \geq 30\%$ is considered to have high level of DNA fragmentation and very poor pregnancy outcomes.
- ▶ Sperms are divided into different populations depending on green and red fluorescence intensities which are shown in Figure as below:



- Region 1: Intact DNA (Sperms with intact chromatin structure)
- Region 2: Fragmented DNA (Sperms with fragmented chromatin structure).
- Region 3: Debris (Cell debris and interference signals)
- Region 4: High DNA stainability (HDS) represents the percentage of sperm with immature chromatin

Illustration of luminescent sperm populations analyzing by SCSA

7. MATERIAL AND EQUIPMENT REQUIRED NOT PROVIDED WITH THE KIT

Devices	Consumable material
Flow cytometer with 488 nm laser	5 mL polypropylene tubes, 12x75 mm (suitable for flow cytometer) - Micro Pipettes and Micro Pipette Tips - Gloves - Ice or cold block

8. WARNINGS, PRECAUTIONS AND PROCEDURE LIMITATIONS

- Semen samples must be collected, transported, and stored under appropriate procedures and conditions. Improper collection, transportation, or storage of samples can give incorrect test results.
- Make sure that semen sample is completely liquefied before testing since semen sample with delayed liquefaction or high viscosity will affect the accuracy of the analytical results and may cause the clog of flow cytometer.
- The test must be completed within 30 minutes after adding the mixture of Reagent C and Reagent D. If the sample is not tested immediately, kept at 2-8°C, protected from light.
- Wear gloves during an entire process.
- Do not use an expired test kit.
- Patient samples and related items must be handled as a source of infection.
- Analytical steps and precautions must be strictly followed to obtain accurate results.
- The test kit must be used by trained users on flow cytometry.
- The test is for screening purposes only.
- Identified clinical diagnoses are performed in combination with the patient's other symptoms and signs, and in conjunction with other test methods.
- Maintenance of flow cytometer according to the manufacturer's instructions, so that the machine works in the best condition.
- Limitations: Do not use the kit for the semen sample with low density, small volume, especially which centrifuged and washed with density is less than a sperm concentration of 0.5×10^6 per mL.

9. EXPLANATION OF SYMBOLS

Symbols	Explanation
	Manufacturer
	Instructions for Use
	Contains sufficient amount for n-tests
	Catalogue number
	Lot number
	Expiry date
	Temperature limitation

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