Instructions for Sperm DNA Fragmentation Detection Kit

(Sperm Chromatin Dispersion Method)

【Product Name】

Sperm DNA Fragmentation Detection Kit (Sperm Chromatin Dispersion Method)

【Specification】

10 Tests/Kit, 20 Tests/Kit

【Intended Use】

The kit is used to detect the ratio of adult male sperm DNA fragmentation in vitro, which reflects the degree of sperm DNA integrity.

【Principle of the method】

The sperm suspension was embedded in the agarose matrix and then placed to the pretreated slide. After acid denaturation, the DNA in the head of the sperm is double-stranded to single-stranded DNA. The sperm cells are cleaved by lysis solution, the nuclear proteins are removed, and then stained.. The results are assessed by microscopy method: the halos correspond to loose chromatin structure attached to the residual nuclear structure in sperm with intact DNA, whereas when DNA is fragmented, dispersion does not develop or is minimal. The integrity of DNA from spermatozoa can be judged according to the existence and size of the dispersion halo, and the integrity of sperm nucleus can be evaluated.

【Main Components】

No.	Main components	Specification		Description
		10 Tests/Kit	20 Tests/Kit	
1	Easy-melting agarose tubes	10 tubes	20 tubes	Low melting point aueous agarose, 1%
2	Pretreated slides	10 pieces	20 pieces	Coated with 0.7% agarose
3	Denaturant solution	20ml×1vial	40ml×1vial	HCl, 0.08N
4	Lysis solution	20ml×1 vial	40ml×1 vial	DTT, 1%
5	Staining solution A	10ml×1vial	20ml×1vial	Wright-Giemsa stain
6	Staining solution B	10ml×1vial	20ml×1vial	PBS, 0.01M

【Storage and Stability】

The kit is stable for 12 months stored at 2~8°C away from light, and can be stable for 3 months at 2-8°C after opening .

【Materials Required (but not provided)】

70% ethanol, ethanol absolute, coverslips, optical microscope, 2~8°C medical refrigerator, 70~100°C dry heater or water bath, 37°C thermostat water bath.

【Specimen Collection and Handling】

1. Collect semen specimens by masturbation or sexual intercourse with a special condom after an abstinence period of 2~7 days.

2. Seminal plasma immediately used for detection after liquefaction, if the seminal plasma sample cannot be detected immediately, stored at -20℃.

【Procedure】

1. Preparations

1.1 Ethanol of gradient concentrations(self-made): dilute absolute ethanol to a certain amount of distilled water to prepare 70% ethanol and absolute ethanol.

1.2 Put the easy-melting agarose tube in a 70~100 ℃ water bath for 1~2 minutes until the agaroses are completely dissolved, then put the easy-melting agarose tube in a 37℃ water bath for use (transfer from 70~100 ℃ to 37℃ for at least 5 minutes of equilibrium until the temperature is constant).

1.3 Adjust the room temperature to 20~28℃ before the experiment.

2. Experiment procedure

2.1 Adjust the the concentration of semen samples to 5~10×10^6/mL with normal saline, then pipette 60μL of sperm suspensions into a equilibrated easy-melting agarose tube and mix thoroughly, and incubated at 37℃ for later use.

2.2 Add 30μl of the low melting point agarose suspension containing sperm to a pretreated slide placed horizontally, and covered with a coverslip gently (without pressing), avoiding air bubbles formation.

2.3 Place the slide in a fridge at 2~8°C for 4 minutes, keeping the slide horizontally throughout the entire process. Take the slide out of the fridge and remove the coverslip by sliding it off gently. Apply Denaturant Solution on the slide and make sure it is fully reacted for 6 minutes.

2.4 Pour out the Denaturant Solution, and use the filter paper to absorb the residual liquid on the back and side edges of the slide (do not touch the specimen area). Apply Lysis Solution on the slide and make sure it is fully reacted for 10 minutes.

2.5 Pour out the Lysis Solution, and use the filter paper to absorb the residual liquid on the back and side edges of the slide (do not touch the specimen area). Then dehydrate the slide by immersing with 70% ethanol and absolute ethanol for 2 minutes each.

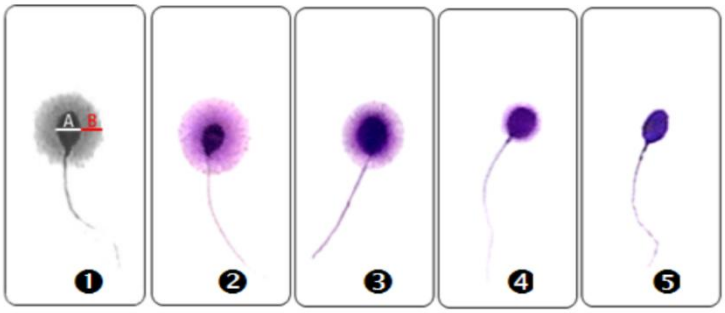
2.6 After dehydration, allow the slides to air dry.

2.7 Mix Staining Solution A and B in a ratio of 1:1. Apply the mixed dye on the slide make sure these are fully immersed. Incubate for 3 minutes. Then, rinse the slide with running distilled water to wash off the stain.

2.8 Allow the slide to dry at temperature. Observe and count under optical microscopy.

【Calculation】

The halos of the spermatozoa in the samples can be classified according to the ratio of the halo (B) to the transverse diameter of the sperm head (A), it is divided into five grades: large, medium, small, without halo and without halo-degraded (As shown below).



① Halo width (B) / the minor diameter of the core(A): B/A ≥ 2/3 is a large halo;

② Halo width (B) / the minor diameter of the core (A): ≥ 1/4, but ≤2/3 is a medium halo;

③ Halo width (B) / the minor diameter of the core(A): B/A ≤ 1/4 is a small halo;

④ Sperm without halo is observed without halo;

⑤ Sperm with no halo and present a core irregularly or weakly stained.

Observe them under a optical microscope with 400× or 1000× lens. Count 400 spermatozoa, and calculating the percentage of abnormal sperm with small halo, without halo or without halo-degraded.

【Reference Interval】

Normal reference value: sperm DNA fragmentation ratio < 25%.

【Result Interpretation】

Temperature of Easy-melting agarose tubes should be balanced to 37℃ before adding samples, in case sperm damage caused by high temperatures (false positives). Prolongation of denaturanting and lysising time can lead to lower sperm DNA fragmentation rate (false negative).

All sperm present with halo or without halo in the same batch of specimens should be suspected to be unreliable result. Find the cause before new test.

【Limitations】

For a same sample, the evaluation and judgment of the experimental results need to be combined with the clinical and laboratory test results in order to obtain a more objective experimental report.

【Performance characteristics】

Staining effect: The morphology of sperm cells is clear, the nucleus staining is clearly distinguished from the halo staining, and the size of the halo is not completely consistent.

【Caution】

1. The kit is only used for in vitro diagnosis, and the test results are for clinical reference only.
2. A suitable and stable reaction temperature (20~28°C) is the key to the success of the experiment. It is vital to abide by the experimental procedure strictly, and the denaturation time and lysis time are very important for the success of the experiment.
3. Care should be taken to avoid contact with skin or eyes, and to prevent inhalation. If denaturant or lysis solution contacts with skin or spills into eyes, rinse with plenty of water and get medical treatment immediately.
4. After denaturation, the morphology of all kinds of round cells in semen change greatly, which is easy to be confused with sperm without tail structure. Therefore, it is necessary to distinguish them and do not count in the calculation of results.
5. Please cover the reagent quickly and keep it sealed to avoid volatilization after using.
6. All patient samples should be treated as potentially infectious. Sample handling and waste disposal must meet local relevant regulatory requirements to avoid environmental pollution.

【Symbols Explanation】

Symbol	Symbol Note	Symbol	Symbol Note
	Manufacturer	IVD	In vitro diagnostic medical device
	Date of manufacture	LOT	Batch code
EC REP	Authorized representative in the European Community	REF	Catalogue Number
CE	CE mark		Use-by Date
	Consult instructions for use		Temperature Limit

Symbol	Symbol Note	Symbol	Symbol Note
	Caution		Contains sufficient for <n> tests

【References】

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【Revision History】

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