Instructions for Sperm Nuclear Staining Solution (Acridine Orange Method)

[Product Name]

Sperm Nuclear Staining Solution (Acridine Orange Method)

[Package Specification]

10 tests/kit, 20 tests/kit

[Intended Use]

Used for staining the sperm nuclear cells of adult males.

[Test Principle]

The single-stranded DNA formed by acid treatment of damaged sperm chromatin will produce red fluorescence when combined with the acridine orange, while normal sperm chromatin can maintain the intact DNA double-stranded structure and will produce green fluorescence when combined with acridine orange.

[Main Component]

No.	Components	Specification		Component
		10 tests/kit	20 tests/kit	
1	Staining Buffer	15mL×1	30mL×1	Contains citric acid
2	Fixation Solution	15mL×1	30mL×1	Contains HCl
3	AO stock solution	0.25mL×1	0.5mL×1	Contains acridine orange
4	Empty brown vial	8mL×1	8mL×1	/

[Storage Conditions and Expiration Date]

Transported in ice bags, and can be stored at 2~8℃ for 12 months.It is valid for 30 days after being opened and stored at 2~8℃.

[Applicable Instruments]

None.

[Sample Requirements]

1. Collect semen from 2-7 days of abstinence by masturbation in a sampling cup, with attention to complete collection.

2. Freshly liquefied semen specimens.

[Test Method]

1. Mix the staining buffer with the AO stock solution in the volume ratio of 1000:6 (e.g., dilute 60 μl of AO stock solution with 10 mL of staining buffer) to prepare the staining solution for use (store it away from light at 2-8°C for 2 weeks after preparation).

2. Prepare the smear of semen specimen using the thinning technique.

3. After the smear is slightly dry (about 20s), add drops of fixation solution to cover the smear and fix it for 30 seconds.

4. Drain the smear by standing it on absorbent paper (1-2 min).

5. Cover the slide with drops of the staining solution prepared in step (1) and stain for 5 minutes protected from light.

6. Rinse the smear with purified water stream and dry it slightly.

7. Observe the luminescence in the nucleus region of the cells at 400X or 1000X under 480 nm of fluorescence microscope excitation.

[Result Calculation]

Count the number of green and red sperm among 200 sperm under fluorescence microscope, where green is DNA double-stranded sperm and red is DNA single-stranded sperm. Calculate the percentage of DNA single-stranded sperm in red fluorescence.

[Reference Value]

Single-stranded DNA sperm <10% is normal.

[Test Result Explanation]

When sperm samples are acid-treated and the nuclei are stained with acridine orange, the DNA single strand of abnormal chromatin will produce red fluorescence when bound to the acridine orange; the DNA of normal chromatin will produce green fluorescence when bound to acridine orange, and if the proportion of red light (value) increases, it indicates an increase in abnormal chromatin structure.

[Method Limitations]

The morphology of the sperm after staining must be observed within 10 min.

[Product Performance Index]

1. pH: The pH of the staining buffer is 5.8-6.1.

2. Staining effect: abnormal single-stranded DNA sperm will produce red fluorescence and normal double-stranded DNA sperm will produce green fluorescence under fluorescence microscope.

Caution

1. Since all samples from the subject should be considered as potential sources of infection, samples and reagents after testing must be disposed of as medical waste to avoid contamination of the environment.

2. This kit is an in vitro diagnostic medical device and the test results are for clinical reference only. The clinical diagnosis of the patient should be considered in conjunction with his or her symptoms, signs, medical history, other laboratory tests and treatment response.

3. For personal safety reasons, please use disposable gloves and handle with care when processing potentially infectious samples (e.g., hepatitis, HIV, etc.).

4. The hydrochloric acid contained in the kit is chemically hazardous and should be avoided by contact or inhalation.

5. The user of the product must have the professional qualifications required for inspection personnel.

6. The product should be used before the expiration date to ensure its effectiveness.

[Symbol Explanation]

Symbol	Symbol Note	Symbol	Symbol Note
	Manufacturer		In vitro diagnostic medical device
	Date of manufacture		Batch code
	Authorized representative in the European Community		Catalogue Number
	CE mark		Use-by Date

Symbol	Symbol Note	Symbol	Symbol Note
	Consult instructions for use		Temperature Limit
	Caution		Contains sufficient for <n> tests

[References]

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4. Payne JF, Raburn DJ, Couchman GM, Price TM, Jamison MG, Walmer DK. Redefine the relationship between sperm deoxyribonucleic acid fragmentation as measured by the sperm chromation structure assay and outcomes of assisted reproductive techniques. Ferility and Sterility 2005,84:356-64.

5. Kazim R, Chohan, Jeanine T, Griffin, et al. Comparison of Chromatin Assay for DNA fragmentation evaluation in human sperm. Journal of Andrology 2006, 27(1):53-59.

[Essential Information]

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[Revision History]

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