Instructions for Staining Solution for the Maturity of Spermatozoan Nucleoprotein (Aniline Blue-Eosin Staining)

[Product Name]

Staining Solution for the Maturity of Spermatozoan Nucleoprotein (Aniline Blue-Eosin Staining)

[Package Specification]

20 tests/kit

[Intended Use]

It is mainly used for staining of spermatozoan nucleoproteins in adult males.

[Test Principle]

During the maturation of sperm, the histotype conversion of basic proteins bound to the DNA of the sperm nucleus will occur. Histones rich in lysine residues will be gradually replaced by protamine rich in arginine and cystine residues. Under acidic conditions, aniline blue will bind to lysine residues to produce a blue or violet-blue compound, thus indicating the presence of proteins rich in lysine residues, while sperm with mature nucleoproteins will be stained to red.

[Main Component]

Product composition	Specification	Main ingredient
Concentrated Washing Solution (10×)	10 ml×1	Contains sodium dihydrogen phosphate
Adhesive Fluid	20 ml×1	Contains gelatin
Fixation Solution	3 ml×1	Contains methanol
Staining Solution	3 ml×1	Contains aniline blue
Concentrated Eluate (5×)	50 ml×1	Contains sodium chloride
Counterstain	3 ml×1	Contains Eosin Y
Customized Slides	8 wells×10 slides	/

The reagent or solution in the kit can be interchanged among different batches

[Storage Conditions and Expiration Date]

Transported in ice bags, and can be stored protected from light at 2-8°C for 12 months; it can be used for 15 days after the vial is opened and stored at 2-8°C.

[Applicable Instruments]

None..

[Sample Requirements]

1. Subjects are required to abstain from sexual intercourse for 2-7 days and all semen specimens should be retained by masturbation or by intercourse using a special semen collection sleeve.
2. Freshly liquefied semen, or semen specimens stored at -20°C or below for one week.

[Test Method]

1. Sample preparation

1)Washing solution preparation: Dilute the concentrated washing solution by 10 times of purified water (e.g., add purified water to 100 ml for 10 ml of concentrated washing solution) and store at 2-8°C.

2)Eluate preparation: Dilute the concentrated eluate by 5 times of purified water (e.g., add purified water to 50ml for 10 ml of concentrated eluate) and store at 2-8°C.

2. Operation method

1)Liquefy and count the semen, and adjust the sperm concentration to 40 x 10^6/ml with washing solution.

2)Transfer 1 ml of the above adjusted semen to a cone-bottom centrifuge tube, centrifuge at 1000g for 10 minutes, and discard the supernatant. Add 1 ml of washing solution and mix well. Centrifuge at 1000g for 3 minutes, discard the supernatant, and repeat the washing 3 times in this manner.

3)After discarding the supernatant after the last centrifugation, add 1 ml of adhesive fluid.

4)Take 5 μl for smearing (the smearing area must be full of smearing window) and dry it naturally.

5)Add drops of fixation solution to cover the smearing window for 90 seconds of fixation and shake off the fixation solution.

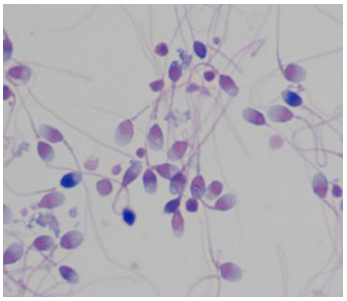
6)Rinse the back of the slide gently with running water 7 times (1 second each time), shake off the water on the surface of the slide (note: do not dry the tissue slide), add drops of dye to cover the smearing area and stain for 5 min.

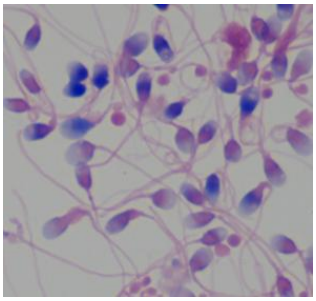
7)Rinse the back of the slide slowly with running water 7 times (1 second each time), then immerse the slide into the eluent for 5 minutes for accurate decolorization, or until no blue color is visible on the slide. Immediately shake off the decolorizing solution and rinse the back of the slide 7 times with running water (1 second each time).

8)Add 2-4 drops of counterstain in the smearing area and stain for 5 min at room temperature.

9)Rinse the back of the slide gently with running water 7 times (1 second each time), shake off the water adhered to the surface of the slide and dry it quickly with cold air or dry in air.

10)Count 200 sperms with a General Optical Microscope with objective 100× oil lens, and the head of immature sperms is blue or purple-blue. Count the number of sperms with head stained blue or purple-blue (sperms with immature nucleoprotein) and calculate the percentage (%).





[Reference Value]

Normal reference value recommended by the World Health Organization (WHO): Sperms with head stained blue or purple-blue ≤ 30% (WHO).

[Test Result Explanation]

1. If the sperm concentration of the specimen is lower or higher than 40 x 10^6/ml, the sperm concentration can be adjusted by washing and then concentrating or diluting.

2. When observing the results under the microscope, the central area of the slide with uniform sperm distribution should be selected for counting. If the sperm appear in clumps are stained blue or purple-blue, they should not be counted as positive sperm (if the sperm suspension is not sufficiently liquefied or unevenly distributed, it will cause local sperm superimposition and making it inappropriate to be decolorized).

[Method Limitations]

Staining Solution for the Maturity of Spermatozoan Nucleoprotein is an In vitro diagnostic medical device and the test results are for clinical reference only. The clinical management of the patient should be considered in conjunction with his or her symptoms/signs, medical history, other laboratory tests and treatment response.

[Product Performance Index]

1. pH value

The pH of the concentrated washing solution is 7.2-7.6.

2. Staining effect

The head of immature sperm is blue or purple-blue, and the head of mature sperm is red or purple-red.

Caution

1. The slides supplied with the kit should be equilibrated to room temperature after being taken out of the refrigerator before opening, and the slides should be kept sealed for a long time, being careful not to contact with the smearing area to avoid contamination of the smearing area.

2. The thickness of the sperm smear and the sperm concentration have a certain influence on the judgment of the results. To ensure the accuracy and good reproducibility of the test results, the sperm concentration should be controlled.

3. Note that excessive sperm loss may occur during sperm washing due to the following reasons: (i) low relative centrifugal force; (ii) excessive short time of centrifugation; (iii) inadvertent removal of sperm precipitate along with the supernatant.

4. The sperm suspension should be fully liquefied before smearing, and the sperm suspension should be evenly spread over the entire smearing window when smearing, and should be placed horizontally to dry after smearing to avoid overlapping of local sperm due to the sperm suspension tending to one side, resulting in uneven decolorization.

5. The time of decolorization should be noted, as excessive time can lead to excessive decolorization of weakly positive sperm to cause false negative results. Short decolorization time can lead to excessive darkness in the background of the slide, which will make the results more difficult to judge.

6. The eluent should be washed off the slide surface with tap water immediately after the decolorization is finished, so as to avoid persistent decolorization leading to prolonged time. After rinsing the decolorizing solution with tap water, try to shake off the water accumulated on the surface of the slide, then put the slide up and blow dry or air dry quickly (avoiding the continued decolorization due to the long retention of tap water in the tissue slides).

7. When washing tissue slides with tap water, a slow flow of water is required and the smearing area should not be rinsed squarely. If the quality of tap water used in the laboratory is poor, please use purified water to replace the tap water.

8. 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.

9. Methanol and glacial acetic acid contained in the kit are chemically hazardous and should be avoided in contact with the skin and eyes.

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

11. 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
	Consult instructions for use		Temperature Limit
	Caution		Contains sufficient for <n> tests

[References]

1. Xiong Chengliang, Wu Mingzhang, Liu Jihong, Huang Yufeng. Human Spermatology, Hubei Science and Technology Press, 2002: 429-432.

2. Guo Yinglu, Hu Liquan. Andrology, People's Medical Publishing House, 2004.

[Essential Information]

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CE mark

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[Revision History]

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