Sperm Nuclear Protein Staining Solution

(Aniline Blue Method)

S1511659

Storage: 2-8°C. Room Temperature. Store in the dark.
Shipping: Shipped with ice packs.

Introduction:

Under normal conditions, the basic proteins (nuclear proteins) bound to sperm nucleus DNA undergo a natural maturation process from histones to protamines. The mature protamines provide special protection for sperm genes (DNA). The gradual replacement of histones by protamines is referred to as sperm nucleoprotein transition, which has important physiological significance. The sperm nucleus carries all the paternal genetic information, and these genes can only be expressed after fertilization. Before fertilization, sperm genes are tightly condensed under the special protection of protamines, with no DNA transcription. However, abnormal nucleoprotein transition can lead to male infertility or early embryonic death and miscarriage. The mechanisms are as follows: ① Sperm DNA becomes unstable and susceptible to damage, making conception difficult; ② Once fertilized, the sperm nucleus cannot decondense normally due to abnormal nucleoprotein transition, thus interfering with the fusion of male and female pronuclei; ③ The embryo fails to develop normally, resulting in embryonic death and miscarriage. Therefore, the amount of histones serves as an important indicator of sperm maturity.

Sperm Nucleoprotein Staining Solution (Aniline Blue Method). Under acidic conditions, aniline blue can specifically bind to lysine residues rich in sperm nuclear histones to form a violet-blue compound. The maturity of spermatozoa is judged by the intensity of staining.This reagent is for research use only and is not intended for clinical diagnosis or other purposes.

Product Components and Storage Conditions:

S1511659	Component	20T	Storage
S1511659A	10× Wash Buffer Concentrate	10mL	RT
S1511659B	Fixative Solution	10mL	2-8℃. Store in the dark.
S1511659C	Aniline Blue Stain	10mL	RT

Materials Required:

1. Distilled water, fresh semen sample, graded ethanol, xylene or eco-friendly dewaxing solution, eco-friendly clearing solution, neutral balsam.
2. (User-supplied reagents) Eosin staining solution, destaining solution.

3. EP tubes, pipettes or pipettors, centrifuge, incubator, adhesive microscope slides.

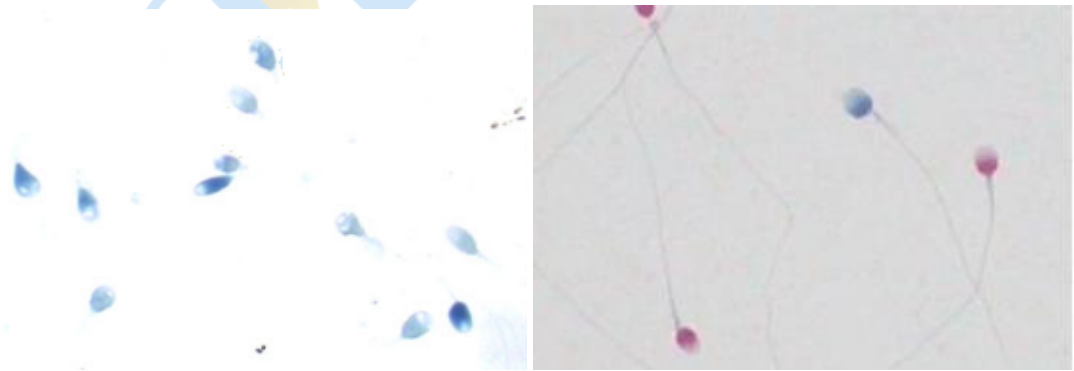
Protocol (For Reference Only):

1. Prepare working wash solution: Dilute the 10× concentrated wash buffer with distilled water at a ratio of 1:10 to obtain the working wash solution.
2. Place the fresh semen sample in an incubator at 37 °C or at room temperature until completely liquefied.
3. Transfer 0.2–0.5 mL of the liquefied semen into an EP tube, add 1–1.5 mL working wash solution, and mix gently by repeated pipetting. Centrifuge at 2000 rpm for 5 min at room temperature, discard the supernatant, and retain the sperm pellet at the bottom. Repeat this step 3 times.
4. Add approximately 0.1–0.2 mL working wash solution to the EP tube from Step 3 to prepare a mixed sperm suspension.
5. Take 15 μL of the prepared sperm suspension and spread evenly onto an adhesive microscope slide; allow to air-dry.
6. Add 2–3 drops of fixative solution onto the sperm smear, fix at room temperature for 5–15 min, rinse with distilled water for 5–10 min, and discard excess water.
7. Add 2–4 drops of aniline blue staining solution onto the smear, stain at room temperature for 5 min, rinse with distilled water for 5 min, and discard excess water.
8. (Optional) Insert the slide vertically into a reaction vessel containing destaining solution, fully immersing the smear area. Incubate at room temperature for 2–5 min. Rinse with distilled water for 1–5 min and discard excess water. Add 2–4 drops of counterstain onto the smear, stain at room temperature for 2–5 min, then rinse with distilled water for 1–5 min.
9. Dry the slide quickly and examine microscopically. For long-term preservation, dehydrate the slide sequentially in 70%, 80%, 95%, and 100% ethanol for 2 min each, clear in xylene or dewaxing/clearing solution, and mount with neutral balsam.

Staining Results:

Sperm with immature nucleoprotein: violet blue or dark blue.

Sperm with mature nucleoprotein: light blue or colorless (red after eosin counterstaining)



Precautions:

1. When rinsing the slides with distilled water, control the flow rate carefully to avoid washing off the sperm on the smear area.
2. When adding the staining solution, ensure it fully covers the smear area and mix evenly by pipetting.
3. After discarding excess water, prevent the smear from drying out.
4. For your safety and health, please wear a lab coat and disposable gloves during operation.