Sperm Nuclear DNA Staining Solution (AO Method)

S1511580

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

Introduction:

Under normal conditions, the basic proteins (nuclear proteins) bound to sperm nuclear DNA undergo a natural maturation process from histones to protamines. The mature protamines exert a special protective effect on 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 activity. However, abnormal nucleoprotein transition can lead to male infertility, early embryonic death or miscarriage. The mechanisms are as follows: ①Sperm DNA becomes unstable and susceptible to damage, resulting in difficulty in fertilization; ②Once fertilized, the sperm nucleus cannot decondense normally due to abnormal nucleoprotein composition, thus interfering with the fusion of male and female pronuclei; ③The embryo fails to develop normally, leading to embryonic death and miscarriage. In normal sperm, the nucleus accounts for approximately 65% of the sperm head and is composed of DNA bound with proteins.Sperm Nuclear DNA Staining Solution (AO Method)Its working principle is that the fluorescent dye acridine orange emits green fluorescence when bound to double-stranded DNA, and red or yellow fluorescence when bound to single-stranded DNA. Generally, only sperm with double-stranded DNA are capable of fertilization. This reagent is for research use only and is not intended for clinical diagnosis or other purposes.

Product Components and Storage Conditions:

S1511580	Component	20T	Storage
S1511580A	Wash Buffer	100mL	RT
S1511580B	Fixative Solution	50mL	RT
S1511580C	AO Stain	1mL	2-8℃. Store in the dark.
S1511580D	AO Buffer	5mL	2-8℃
S1511580E	Anti-Fade Mounting Medium	5mL	2-8℃

Materials Required:

1. Distilled water, fresh semen sample.

2. EP tubes or centrifuge tubes, incubator, adhesive microscope slides.

Protocol (For Reference Only):

1. Place the fresh semen sample in an incubator at 37 °C or at room temperature until completely liquefied.
2. Transfer 0.2–0.5 mL of liquefied semen into an EP tube, add 1–1.5 mL wash buffer, and mix by repeated pipetting. Centrifuge at 2000 rpm for 5–10 min at room temperature, discard the supernatant, and retain the sperm pellet. Repeat 1–2 times.
3. Add 0.05–0.2 mL wash buffer to the sperm pellet to prepare a sperm suspension.
4. Apply 15–100 μL of the sperm suspension evenly onto an adhesive microscope slide and air-dry.
5. Add 2–3 drops of fixative solution onto the smear, fix at room temperature for 10–15 min, rinse briefly with distilled water, and decant excess water.
6. Prepare AO working solution: mix AO stain and AO buffer at a ratio of 1:4. Prepare fresh before use and do not store.
7. Add 2–4 drops of AO working solution onto the smear, stain at room temperature for 5 min, rinse briefly with distilled water, and decant excess water.
8. Blow-dry or air-dry the slide, add 2–4 drops of anti-fade mounting medium, and observe under a fluorescence microscope.

Staining Results:

Nuclear DNA: green fluorescence.

Precautions:

1. When rinsing slides with distilled water, control the flow rate to avoid washing off sperm from the smear.
2. After decanting excess water, prevent the smear from becoming overly dry.
3. AO working solution must be prepared fresh and used immediately; otherwise, fluorescence will fade. If fluorescence is significantly reduced, maintain the ratio of AO stain:AO buffer = 1:4 for optimal results.
4. For your safety and health, wear a lab coat and disposable gloves during operation.