

SPERM HY-LITER™

SPERM HY-LITER PLUS™ Staining Protocol



Kit Provided Solutions:

FIXATIVE Solution	white bottle cap
SAMPLE PREPARATION Solution	yellow bottle cap (<i>addition of DTT required before use</i>)
BLOCKING Solution	red bottle cap
SPERM HEAD STAINING Solution	green bottle cap
MOUNTING Media	blue bottle cap
WASH Buffer 10X Stock	square 250 ml bottle (<i>dilution required before use</i>)

User-prepared solutions:

1X Wash Solution

Prepare 1X wash solution from provided 10X Stock: dilute 1:10 with H₂O into a convenient wash/squirt bottle.

Sample Preparation Solution + DTT

Prepare Sample Preparation + DTT daily before use: for each sample window to be stained, add 1 µl of 1 M DTT to two drops of Sample Preparation Solution (yellow bottle cap) in a microcentrifuge tube and mix thoroughly.

Procedure:

- 1. Fixation:** Add 2 drops of **FIXATIVE Solution (white bottle cap)** to each circular sample window. Incubate at room temperature for 10 minutes.

Wash: Use a wash bottle to **gently** rinse each sample window with approximately 2-3 mL of 1X wash buffer. Vigorous washing or rinsing is **not** required. After the wash step, use a corner of a paper towel or a lab wipe to wick away the residual wash buffer in the sample window.

- 2. Sample Preparation:** Pipette user-prepared **SAMPLE PREPARATION Solution + DTT (~ 80 µl)** to each circular sample window. Incubate at room temperature for 30 minutes.

Wash: Wash slide as described above.

- 3. Block:** Add 2 drops of **BLOCKING Solution (red bottle cap)** to each circular sample window. Incubate at room temperature for 30 minutes.

Wash: Wash slide as described above.

- 4. Stain:** Add 2 drops of **SPERM HEAD STAINING Solution (green bottle cap)** to each circular sample window. Incubate at room temperature for 30 minutes.

Wash: Wash slide as described above.

- 5. OPTIONAL - Mount:** Add one drop of **MOUNTING Media (blue bottle cap)** to each circular sample window. Gently place provided cover slip over each sample window. Place slide between two small stacks of paper towels and gently press down to position coverslip and remove excess mounting media. Mounting media will semi-harden after 20 minutes at room temperature. Coverslips may be stabilized by outlining with clear nail polish. Slides may be visualized immediately and are stable for days.

- 6. Visualize:** Stained slides must be visualized using a fluorescence microscope fitted with appropriate filters. Cell nuclei, including epithelial and sperm, can be visualized using DAPI-compatible filters. Human sperm heads can be visualized using fluorescein or Alexa 488 compatible filters. Slides may be scanned at 10x, 20x, 40x or 100x at the operator's discretion.

Additional Suggested Protocol:

Extract Preparation:

Remove the fabric cutting, swab batting or the entire swab head using either a clean scalpel or a clean pair of scissors. Place cutting, batting or swab head in a microfuge tube.

Incubate the swab batting, swab head or cutting in PBS at room temperature for one hour. Laboratory personnel should use a volume of soak solution compatible with their own methods.

Remove swab batting, swab head or cutting from tube using Spin-Eze™, tweezers or similar, and pellet cells by centrifugation for 1 min at 13,000 X RPM.

Remove supernatant with fine-tipped pipette or similar.

Re-suspend pellet in 25-100 µl of PBS.

Remove ~10 µl of the re-suspended cells and place in a circular sample window of a **SPERM HY-LITER™** slide. Printed side of slide should be facing up.

Spread the sample evenly over the sample window using a pipette tip.

Allow the sample to air dry until no liquid remains in the sample window, approximately 15 minutes. Drying can be expedited by placing the slide in a 37°C incubator, under a desk light, or on a hot plate set to the **lowest** setting.

Dried slides may be stored for several weeks or processed immediately for **SPERM HY-LITER™** staining.

NOT FOR IN VITRO DIAGNOSTIC USE

Is semen present?

RSID™-SEMEN

Was there oral contact?

RSID™-SALIVA

Was there a struggle?

RSID™-BLOOD

Are sperm present?

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