

# SPERM HY-LITER™

## SPERM HY-LITER™ Staining Protocol

This protocol is intended for use in conjunction 11mm well, (slide cat. #: 9111-25/26, 9211-25/26).  
For use with slides having wells of another size, 6 mm or 8 mm (slide cat. #: 9106-25/26, or 9408-25/26),  
Refer to Additional Suggested Protocols.



### Kit Provided Solutions:

<b>FIXATIVE Solution</b>	white bottle cap
<b>SAMPLE PREPARATION Solution</b>	yellow bottle cap ( <i>addition of DTT required before use</i> )
<b>BLOCKING Solution</b>	red bottle cap
<b>SPERM HEAD STAINING Solution</b>	green bottle cap
<b>MOUNTING Media</b>	blue bottle cap
<b>WASH Buffer 10X Stock</b>	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 H<sub>2</sub>O 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  $\mu$ l of 1 M DTT to two drops of Sample Preparation Solution (yellow bottle cap) in a microcentrifuge tube and mix thoroughly. Refer to the DTT solution protocol on the reverse. It has been observed that increased amounts of DTT can produce improved fluorescent signal in samples demonstrating weak staining. Analysts, at their discretion, may increase the amount of 1M DTT added to the Sample Preparation Buffer up to 10X, i.e., up to 10  $\mu$ l per 2 drops of sample preparation solution.

### 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:** [*DTT must be added to this solution prior to use! Please see User-Prepared Solutions above.*] Pipette user-prepared **SAMPLE PREPARATION Solution + DTT** (~ 80  $\mu$ l) 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.

**\*Please note that warm and humid conditions can slow or prevent hardening of the mounting media.** This will not affect the quality of the staining.

**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 Protocols:

### 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. Incubation in a sonicator water bath for 20 min will improve release of cells from cuttings and swabs.
- 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.
- Dried slides may be stored for several weeks or processed immediately for **SPERM HY-LITER™** staining.

### Protocol for non 11 mm window slides:

For slides with 6 mm or 8 mm sample windows, reduce number of drops used per window from 2 drops to 1 drop. All solutions remain unchanged, including Sample Preparation + DTT.

### DTT solution

To make 1M DTT pH8.0

	1 ml	10 ml	100 ml
DTT	0.154 g	1.54 g	15.4 g
H <sub>2</sub> O	~0.7 ml	~7 ml	~70 ml
1M KOH	0.11 ml	1.1 ml	11 ml

Confirm that the solution is at pH 8.0, add H<sub>2</sub>O up to the indicated final volume, aliquot and freeze. Aliquots may be frozen and thawed twice before discarding.

### Integration of KPIC staining and SPERM HY-LITER™:

**Archived slides previously stained with KPIC.** Mounting media, if present, must be removed before previously prepared slides can be stained with SPERM HY-LITER™. Permouted slides may be soaked in xylene to dissolve the media.

Proceed with SPERM HY-LITER™ staining as per protocol. Archived KPIC slides will demonstrate weaker SPERM HY-LITER™ sperm staining: more intense staining may be observed by increasing the DTT concentration in the Sample Preparation Solution by 10X (i.e., 10 µl of 1M DTT per two drops of Sample Preparation Solution – Yellow Capped bottle).

**To stain freshly prepared slides with KPIC followed by SPERM HY-LITER™.** We recommend that analysts slightly modify their existing procedure by substituting the final ethanol wash in the KPIC protocol with a gentle water rinse. The SPERM HY-LITER™ protocol may then be followed as described.

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NOT FOR IN VITRO DIAGNOSTIC USE