

Independent Forensics  
**SPERM HY-LITER *PLUS*<sup>TM</sup>**  
**Technical Information Sheet**

### INTENDED USE

The new SPERM HY-LITER<sup>TM</sup> kit for the microscopic detection of human sperm is designed for specific, sensitive, reliable and easy detection of human sperm from sexual assault evidence including swabs and smear slides.

The test can detect a single human sperm head in an overwhelming background of epithelial cells.

Sample processing and fluorescent detection of human sperm using SPERM HY-LITER<sup>TM</sup> can be completely integrated into current forensic laboratory procedures for DNA-based analysis, prior to STR testing (see Provided Protocols).

SPERM HY-LITER<sup>TM</sup> is highly specific for human sperm heads such that if a fluorescent signal is observed, an analyst can conclude that human sperm has been detected and that male genetic material is most likely present in the tested sample. SPERM HY-LITER<sup>TM</sup> is completely compatible with DNA-based STR analysis.

This is the first commercially available, specific, confirmatory test for human sperm: it does not rely on morphological characteristics or non-specific staining to positively identify human sperm heads. No other human body fluids or animal semen samples (including blood, urine, and epithelial cells; dog, cat, cow, horse, goat, sheep, pig, and mouse) cross-react with SPERM HY-LITER<sup>TM</sup>. Unlike other commercially available sperm detection kits, SPERM HY-LITER<sup>TM</sup> only stains human sperm heads, provides a bright, highly fluorescent signal from the only sperm structure remaining on sexual assault evidence: the DNA-containing sperm head. SPERM HY-LITER<sup>TM</sup> uses a unique monoclonal antibody specific for human sperm heads in conjunction with a simple, defined protocol to provide a scientifically justifiable identification of human sperm by fluorescent microscopy.

*NOT FOR IN VITRO DIAGNOSTIC USE.*

### Introduction

SPERM HY-LITER<sup>TM</sup> uses a fluorescently tagged anti-human sperm head monoclonal antibody to detect the presence of human sperm heads.

SPERM HY-LITER<sup>TM</sup> is a confirmatory test for human sperm and has numerous advantages over

other methods of sperm detection, including increased sensitivity and specificity. Current identification methods for semen lack discrimination and are by definition, presumptive (provide a basis for continued analysis of the tested exhibit but are not specific for human sperm), and are therefore open to legal and scientific challenge.

### Principle of the Test

SPERM HY-LITER<sup>TM</sup> uses an Alexa 488 derivatized mouse monoclonal antibody to human sperm heads to specifically identify human sperm from sexual assault evidence by fluorescent microscopy. The method requires a fluorescent microscope: processed slides can be visualized on *any* commercial fluorescent microscope fitted with the correct excitation and emission filters. In addition to a human sperm specific reagent, SPERM HY-LITER<sup>TM</sup> incorporates a second fluorescent dye that stains all nuclei present in the sample. Visualization of fluorescent nuclei is not required for sperm detection, but is recommended for both manual and automated sperm searches.

SPERM HY-LITER<sup>TM</sup> requires simple, sequential sample processing using provided solutions to attach, prepare, block and stain microscopic evidence for the detection of human sperm. Practitioners may use their own smear slides or apply extracts to SPERM HY-LITER<sup>TM</sup> slides (SPERM HY-LYTER<sup>TM</sup> slides are specially prepared for efficient attachment of sperm and have defined sample application areas such that consistent results can be achieved by all users). Processed slides may be visualized immediately with or without the addition of mounting media or a coverslip. Laboratories that intend to isolate sperm from SPERM HY-LITER<sup>TM</sup> preparations for DNA-STR analysis might consider leaving their preparations unmounted; mounted coverslips can be removed by soaking in water for several hours.

SPERM HY-LITER<sup>TM</sup> incorporates a fluorescent nucleic acid stain that can be used to locate all cells in the preparation: dual color analysis of SPERM HY-LITER<sup>TM</sup> preparations (DAPI and Fluorescein) can be used as an aid to visualizing crowded preparation and/or with image analysis software to electronically eliminate fluorescent background signals. This additional fluorescent stain is included in anticipation of the widespread use of automated sperm search software and the use of Laser Capture Microdissection methods. It is not required for the detection of human sperm.

## Reagents and Materials Provided

### i) Provided Solutions:

Fixative Solution	store at 2-8°C
Sample Preparation Solution	store at 2-8°C
Blocking Solution	store at 2-8°C
Sperm Head Staining Solution	store at 2-8°C
Mounting Media	store at 2-8°C
Wash Buffer 10X Stock	store at RT

### ii) SPERM HY-LITER™ slides

25, two (2) position masked and coated slides.

### iii) Positive Control Slide (included in sample kits only)

Laboratory slide with either epithelial cells (position #1) or with epithelial cells and human sperm (position #2) prepared using current SPERM HY-LITER™ lot. Store at RT protected from light.

### iv) 50, 18 x 18 glass cover slips. Store at RT.

### v) Staining Protocol and Technical Information Sheet

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## Required User-Prepared Solutions

### i) 1 X Wash Solution

Users must prepare a 1X wash solution from the provided 10X stock - dilute stock 1:10 with laboratory quality H<sub>2</sub>O into a convenient wash/squirt bottle. Store at RT.

### ii) Sample Preparation Solution + DTT

Prepare Sample Preparation Solution + DTT daily before use: for each sample window to be stained, add 1 µl of freshly thawed 1 M DTT to two (2) drops of Sample Preparation Solution (yellow bottle cap) in a microcentrifuge tube, mix thoroughly. Laboratories that do not use 1 M DTT stock solutions should adjust DTT volumes accordingly; final concentration of DTT in Sample Preparation Solution should be ~12 mM.

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## SPERM HY-LITER™ Staining Protocol

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

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

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

Wash. Wash slide as described above: *gently* rinse each sample window with ~2-3 mL of 1X wash buffer.

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

Wash. Wash slide as described above: *gently* rinse each sample window with ~2-3 mL of 1X wash buffer.

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

Wash. Wash slide as described above: *gently* rinse each sample window with ~2-3 mL of 1X wash buffer.

Slides may be visualized immediately, or for better optical and photographic quality, mounted and coverslipped (see below). Processed SPERM HY-LITER™ slides should be stored at room temperature protected from light.

**5. OPTIONAL - Mount:** Add one drop of MOUNTING Solution (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 min at room temperature. Coverslips may be stabilized by outlining with clear nail polish. Slides may be visualized immediately and are stable for 7-10 days.

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## Visualization of Human Sperm Heads

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

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## Specificity

SPERM HY-LITER™ is specific for human sperm heads. No cross-reactivity with epithelial cells, blood cells or animal semen has been observed.

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## Test Sensitivity

The detection limit for SPERM HY-LITER™ used as suggested is one sperm head.

*Manufactured by:*

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