

**Independent Forensics**  
**SPERM HY-LITER™ PI**  
**Technical Information Sheet and Additional**  
**Suggested Protocols**

### INTENDED USE

The SPERM HY-LITER™ PI kit is designed for specific, sensitive, reliable and simple detection of human sperm from sexual assault evidence 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 can be completely integrated into current forensic laboratory procedures for DNA-based analysis, prior to STR testing

The SPERM HY-LITER™ line of kits is the first commercially available, specific, confirmatory test for human sperm: morphological characteristics and non-specific staining methods are **NOT** used to identify human sperm heads. No other human body fluids cross-react. Unlike other commercially available sperm detection kits, SPERM HY-LITER™ kits only stain human sperm *heads*, providing a bright fluorescent signal from the only sperm structure remaining in most sexual assault evidence: the DNA-containing sperm head. SPERM HY-LITER™ kits utilize 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 fluorescence microscopy.

*NOT FOR IN VITRO DIAGNOSTIC USE.*

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#### Introduction

SPERM HY-LITER™ PI uses a fluorescently tagged anti-human sperm head monoclonal antibody to detect the presence of human sperm. Many identification methods for semen are directed toward protein markers in seminal fluid rather than human sperm. Further, cell stains commonly relied upon to identify sperm provide no species information. SPERM HY-LITER™ PI provides specific identification of the human origin of the sample and confirms that male-origin cells are present.

#### Principle of the Test

SPERM HY-LITER™ PI uses an Alexa 488 derivatized mouse monoclonal antibody to human sperm heads to specifically identify human sperm from sexual assault evidence by fluorescence microscopy. The method requires a fluorescence microscope: processed slides must be visualized on a *fluorescence* microscope fitted with the correct excitation and emission filters and light source.

In addition to a human sperm specific reagent, SPERM HY-LITER™ PI incorporates a second fluorescent dye that stains all nuclei present in the sample (propidium iodide, PI). Visualization of fluorescent nuclei is not required for sperm detection, but is recommended for both manual and automated sperm searches.

SPERM HY-LITER™ PI requires simple, sequential sample processing using provided solutions to attach, prepare, block and stain microscopical evidence for the detection of human sperm. Analysts apply extracts to provided slides that are specially prepared for efficient attachment of biological material and have defined sample application areas such that consistent results can be achieved by all users. Processed slides may be visualized immediately. Mounted slides are recommended for optimal visual quality. However, laboratories that intend to isolate sperm from stained preparations for DNA-STR analysis might consider leaving their preparations unmounted. Alternatively, mounted coverslips can be removed by soaking in water.

SPERM HY-LITER™ PI incorporates a fluorescent nucleic acid stain that can be used to locate all cells in the preparation: dual color analysis (PI and Alexa 488) can be used as an aid to visualizing crowded preparations and/or with image analysis software to electronically eliminate fluorescent background signals. The additional fluorescent stain of propidium iodide is included in anticipation of the widespread use of automated sperm search software and the use of Laser Capture Microdissection methods.

#### 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 PI-compatible filters. Human sperm heads can be visualized using fluorescein or Alexa 488 compatible filters. Slides may be scanned at a final magnification of 100x, 200x, or 400x at the operator's discretion.

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#### Specificity

SPERM HY-LITER™ PI is specific for human sperm heads. No cross-reactivity with epithelial nuclei, blood cells or animal semen from horse, bull, sheep, goat, pig, dog, cat, mouse and chimpanzee has been observed. To date, semen from 3 nonhuman primates has been tested: common chimp, Rhesus macaque, and cynomolgus macaque. SPERM HY-LITER™ PI does NOT detect sperm heads from these species.

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## Test Sensitivity

When used as suggested, the detection limit for SPERM HY-LITER™ PI is one human sperm head.

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## Reagents and Materials Required

### 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 RT
Wash Buffer 10X Stock	store at RT

### ii) Staining Protocol

### iii) Technical Information Sheet and Additional Suggested Protocols

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## 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 microcentrifuge tube.

-Incubate the fabric cutting, swab batting, or swab head in PBS at room temperature for one hour with occasional vortexing. 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 pipette tip.

-Allow the sample to air dry until no liquid remains in the sample window, approximately 15 mins.

-Dried slides should be processed immediately for **SPERM HY-LITER™ PI** staining.

## DTT solution

**SPERM HY-LITER™ PI staining is critically dependent on the proper DTT concentration and pH. Use the following recipe to prepare the DTT solution that will be added to the Sample Preparation Buffer**

To make stock 1 M DTT, pH 8.0

	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
1 M KOH	0.11 mL	1.1 mL	11 mL

Confirm that the solution is at pH 8.0 (adjust pH with KOH or HCl if necessary), add H<sub>2</sub>O up to the indicated final volume, aliquot and freeze. Aliquots may be frozen and thawed twice before discarding.

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