

Independent Forensics
SPERM HY-LITER™ PLUS
Technical Information Sheet

INTENDED USE

The new SPERM HY-LITER™ PLUS kits is designed for specific, sensitive, reliable and simple detection of human sperm from sexual assault evidence extract analysis. 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 (see Provided Protocols).

SPERM HY-LITER™ PLUS 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 present in the tested sample.

SPERM HY-LITER™ PLUS is the first commercially available, specific, confirmatory test for human sperm: morphological characteristics and non-specific staining is **NOT** used to identify human sperm heads. No other human body fluids or animal semen samples (including blood, urine, and epithelial cells; dog, cat, bull, horse, goat, sheep, pig, and mouse) cross-react. Unlike other commercially available sperm detection kits, SPERM HY-LITER™ PLUS only stains 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™ PLUS utilizes 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.

Introduction

SPERM HY-LITER™ PLUS uses a fluorescently tagged anti-human sperm head monoclonal antibody to detect the presence of human sperm. The tests are confirmatory for human sperm and have 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 open to legal and scientific challenge.

Principle of the Test

SPERM HY-LITER™ PLUS 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 can be visualized on *any* commercial fluorescence microscope fitted with the correct excitation and emission filters. In addition to a human sperm specific reagent, SPERM HY-LITER™ PLUS 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™ PLUS requires simple, sequential sample processing using provided solutions to attach, prepare, block and stain microscopic evidence for the detection of human sperm. Practitioners apply extracts to provided slides that 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. 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 for several hours.

SPERM HY-LITER™ PLUS incorporates a fluorescent nucleic acid stain that can be used to locate all cells in the preparation: dual color analysis (DAPI and Fluorescein) can be used as an aid to visualizing crowded preparations 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) 25, two (2) position masked slides (SPERM HY-LITER™ PLUS only). Store at RT.

iii) Control Slide: Stained slide with epithelial cells (position #1) and epithelial cell/sperm mixture (position #2). Store at RT protected from light.

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

v) Staining Protocol and Technical Information Sheet

User-Prepared Solutions

i) 1 X Wash Solution

Users must prepare a 1X wash solution from the provided 10X stock – dilute provided stock 1:10 with laboratory quality H₂O 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.

Staining Protocol

1. Fixation: Add 2 drops of FIXATIVE Solution (white bottle cap) to each sample. Incubate at room temperature for 10 min. Wash: Use a wash/ squirt bottle to *gently* rinse each sample with ~2-3 mL of 1X wash buffer. Vigorous or lengthy washing or rinsing is *not* required. After the wash step, use the 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 sample. Incubate at room temperature for 30 min. Wash slide as described above.

3. Block: Add 2 drops of BLOCKING Solution (red bottle cap) to each sample. Incubate at room temperature for 30 min. 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 min. Wash slide as described above.

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

5. OPTIONAL - Mount: Add one drop of MOUNTING Solution (blue bottle cap) and place cover slip over each sample. Place slide between 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 several weeks stored in the dark at room temperature.

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 a final magnification of 100x, 200x, 400x or 1000x at the operator's discretion.

Specificity

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

Test Sensitivity

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

Manufactured by:

 **Independent Forensics**
4600 ROOSEVELT RD., STE. 201 - HILLSIDE, IL 60162
TEL. 866-434-2400 FAX 708-978-5115