SPERM HY-LITER™ *Express*

Staining Protocol

Kit Provided Solutions:

FIXATIVE Solution	**white** bottle cap
SAMPLE PREPARATION Solution	**yellow** bottle cap (*addition of DTT **required** before use)*
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 (*dilution **required** before use*)

*Use the following protocol only with **SPERM HY-LITER™ *Express*** solutions; Do not substitute reagents from the standard kit.

SPERM HY-LITER™ *Express* may be used with several different types of slides, and the following table specifies the number of drops of provided solution that should be used per sample window. The volume of solution is proportional to the size of the sample window /slide area to be stained. Use the indicated number of drops for each step in the protocol [except sample preparation solution (see below)]

Size of sample window diameter	6 mm	8 mm	11 mm	smear slide
IFI cat#	9106-25/26	9408-25/26	9111-25/26	9000-25/26 or regular glass slide
# drops used per sample window	1	1	2	6-12 (use sufficient volume to cover sample)

Determine slide configuration: Determine the diameter of slide sample well and use the indicated number of drops of provided solutions for your slide configuration.

If staining smear slides, a hydrophobic barrier must be applied to the slide around the area to be stained. Depress the tip of ImmEdge Pen™ onto the inside wall of a 1.5 mL microcentrifuge tube, thereby releasing a stream of the pen reagent (100 µL per slide is sufficient) into the tube. Using a disposable pipette tip*, dispense "released pen reagent" to frame the entire sexual assault evidence smear. Let dry prior to staining. ***Note: To prevent cross-contamination between samples use a fresh pipette tip for every slide!**

User-prepared solutions:

1X Wash Buffer
Prepare 1X wash buffer from provided 10X Stock: dilute 1:10 with DD H_2O into a convenient wash/squirt bottle.

Sample Preparation Solution + DTT
Prepare Sample Preparation + DTT daily before use: Calculate number of drops required for the number of sample windows to be stained (refer to table above). Prepare DTT according to the provided DTT solution protocol in "Additional Suggested Protocols" below. It has been observed that increased amounts of DTT can produce improved fluorescent signal in samples demonstrating weak staining. For optimal staining with **SPERM HY-LITER™** *Express,* 10X DTT is recommended (i.e., 5 µL of 1M DTT per drop of sample preparation solution).

Procedure:

A. Prepare user-prepared solutions:
Prepare 1X Wash Buffer and Sample Preparation Solution + DTT

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B. Perform 5-step staining protocol using indicated volumes for the chosen slide configuration.

1. Fixation: Add **FIXATIVE Solution (white bottle cap)** to each sample window. Incubate at room temperature for 5 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*** recommended or required. After the wash step, use a corner of a paper towel or 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! See User-Prepared Solutions above.]* Pipette user-prepared **SAMPLE PREPARATION Solution** + **DTT** onto each sample window. The volume of sample preparation solution + DTT will be determined by the slide being stained. Refer to table below for correct volume.

Size of sample window	6 mm	8 mm	11 mm	SAE smear slide
Volume of Sample Prep + DTT	~20 µL	~40 µL	~80 µL	240 – 320 µL

Incubate at room temperature for 15 minutes.

Wash: Wash slide as described above.

3. Block: Add **BLOCKING Solution (red bottle cap)** to each sample window. Incubate at room temperature for 15 minutes.

Wash: Wash slide as described above.

4. Stain: Add **SPERM HEAD STAINING Solution (green bottle cap)** to each sample window. Incubate at room temperature for 15 minutes.

Wash: Wash slide as described above.

5. Mount: Add one drop of **MOUNTING Media (blue bottle cap)** to each sample window (use three drops for smear slides). Gently place provided cover slip over each sample window. Place slide between a folded paper towel and gently press down to position cover slip and remove excess mounting media. Mounting media will semi-harden after 20 minutes at room temperature*. Slides may be stabilized by outlining the cover slip with clear nail polish. Slides may be visualized immediately and are stable for days. ***Note that warm and humid conditions can slow or prevent hardening of the mounting media.** This will not affect the quality of the staining.

C. 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 using 10x, 20x, 40x or 100x at the operator's discretion.

DTT Solution Recipe

Final Volume:	1 mL	10 mL	100 mL
DTT	0.154 g	1.54 g	15.4 g
H_2O	~0.7 mL	~7 mL	~70 mL
1 M KOH	0.11 mL	1.1 mL	11 mL

Confirm that the final DTT solution is ~pH 8.0. Add DDH_2O to the indicated final volume, aliquot and freeze. Aliquots may be frozen and thawed twice before discarding.

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

INTENDED USE

The SPERM HY-LITER™ *Express* 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.

Introduction

SPERM HY-LITER™ kits use 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™ kits provide specific identification of the human origin of the sample and confirm that male-origin cells are present.

Principle of the Test

SPERM HY-LITER™ *Express* uses a CF™488A 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™ *Express* incorporates a second fluorescent dye that stains all nuclei present in the sample (4',6-diamidino-2-phenylindole, DAPI). Visualization of fluorescent nuclei is not required for sperm detection, but is recommended for both manual and automated sperm searches.

SPERM HY-LITER™ *Express* 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™ *Express* incorporates a fluorescent nucleic acid stain that can be used to locate all cells in the preparation: dual color analysis (DAPI and CF™488A) 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 DAPI 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 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, or 400x at the operator's discretion.

Specificity

The SPERM HY-LITER™ line of kits 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™ *Express* does NOT detect sperm heads from these species.

Test Sensitivity

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

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 2-8°C
Wash Buffer 10X Stock	store at RT

ii) Staining Protocol

iii) Technical Information Sheet and Additional Suggested Protocols

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 processed immediately for **SPERM HY-LITER™** staining.

DTT solution

SPERM HY-LITER™ Express 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_2O	~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_2O up to the indicated final volume, aliquot and freeze. Aliquots may be frozen and thawed twice before discarding.

Manufactured by:

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DNA Testing & Technologies

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