SPERM HY-LITER™ PI

Staining Protocol

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

SPERM HY-LITER™ 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. Analysts, at their discretion, may increase the amount of 1 M DTT added to the Sample Preparation Buffer up to 10X, i.e., up to 5 µL per drop of sample preparation solution.

Procedure:

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

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

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1. Fixation: Add **FIXATIVE Solution (white bottle cap)** to each 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*** 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 30 minutes.

Wash: Wash slide as described above.

3. Block: Add **BLOCKING Solution (clear bottle cap)** to each sample window. Incubate at room temperature for 30 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 30 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 propidium iodide-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

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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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