SPERM HY-LITER™ *SOS*

Solution Staining Protocol

Kit Provided Solutions:

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

User-prepared solutions:

0.1 M Dithriothreitol (DTT) Solution – Use Independent Forensics 1 M DTT or Prepare 1 M DTT according to the provided DTT solution recipe below. Dilute 1 M DTT stock 1:10 in DiH_2O *(i.e., 10 µL 1M DTT + 90 µL DiH_2O).*

1 M DTT Solution Recipe

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

Confirm that the prepared DTT solution is ~pH 8.0. Add DiH_2O to the final volume, aliquot and freeze. Aliquots may be frozen and thawed twice before discarding.

Sample Preparation Solution (SPS)+ DTT - Prepare SPS + DTT daily before use:
Prepare sufficient SPS + DTT master mix for all of your samples, plus 1 extra. Each sample will require 40 µL of sample prep solution; dispense sufficient SP into a tube and remove [(#samples + 1) x 40] µL into a fresh tube.
Add 5 µL of 0.1M stock DTT solution per (sample +1) to complete the SPS + DTT master mix.
For example, if three (3) samples are to be stained, add 4-5 drops Sample Preparation Solution to a tube and dispense 160 µL of SP into a fresh tube. Add 20 µL of 0.1 M DTT to the measured SP solution.

Sperm cells in solution are significantly more sensitive to DTT relative to sperm stained on slides. Accurate concentrations and incubation times are particularly important in the SOS technique.

Staining Procedure:

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

Preparation of extracts:

- Extract swab or fabric cutting in 1.7 mL microcentrifuge tube in 0.5 mL of PBS. Incubate on bench top for 1-2 hrs with occasional mixing.
- Recover cells using Spin basket: transfer the soaked swab head or fabric cutting into a spin basket, place the basket with the substrate into its same 1.7 mL tube. Centrifuge for 5 minutes at max rpm (~13,000 x g).

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Carefully remove supernatant from cell pellet – to avoid disturbing the pellet, leave behind about 50 µL, do not remove all the supernatant. **For staining, use 10-20 µL of resuspended cell pellet for staining in solution.**

Suggested control swabs:
Negative – buccal swab pellet resuspended in 50-100 µL of PBS
Positive – semen cell pellet resuspended in 50 µL of PBS.

- Prepare three controls samples for staining:
A. Buccal only (10 µL of resuspended Buccal cell pellet in a tube)
B. Sperm only (10 µL of resuspended sperm cell pellet in a tube)
C. Buccal + sperm (10 µL each of resuspended buccal and sperm cell pellets).

A. Prepare 0.1 M DTT and Sample Preparation Solution + DTT: DTT stock for protocol on slides is 1 M. ***The DTT stock for in-solution staining is 0.1 M and must be diluted from the 1 M stock.***

B. Steps for Staining In Solution:

1. **Sample Preparation**: Add 45 µL of SPS/DTT solution (prepared as above) to each sample to be stained. Incubate at room temperature for 5 min with occasional gentle mixing.
2. **Block:** Add 1 drop **BLOCKING Solution (red bottle cap)** to each sample. Incubate at room temperature for 5 minutes with occasional mixing.
3. Centrifuge tube for 3 minutes at 7,000 RPM and gently remove most of supernatant (all but ~50 µL) being careful not to disturb the cell pellet.
4. **Stain:** Add 1 drop **SPERM HEAD STAINING Solution (green bottle cap)** to each sample. Resuspend cell pellet by pipetting up and down and incubate at room temperature for 5 minutes.
5. Centrifuge for 3 minutes at 7,000 RPM and gently remove most of supernatant (all but ~50 µL), being careful not to disturb the cell pellet.
6. Resuspend pellet in 10-20 µL PBS and deposit entire volume onto 11 mm sample window of a microscope slide. Allow to completely dry before proceeding to next step.
7. **Mount:** Add one drop of **MOUNTING Media (blue bottle cap)** to each sample window. 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.

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 objectives at the operator's discretion.