

Independent Forensics
Rapid Stain Identification
Of Human Semen (RSID™-Semen)
Provided Protocols

GENERAL GUIDELINES

When possible, stains deposited on fabric or other substrates that can be easily cut, should be dissected to preserve at least half of the questioned stain in order to allow for re-testing. Scissors or scalpels used to cut the underlying substrate of questioned stains should be washed in 95% ethanol and deionized water, and then dried with a fresh Kimwipe before use and between each cutting. Special emphasis should be paid to the hinge region of scissors. We recommend immersing scissors and scalpels sequentially in 95% ethanol and deionized water between uses. Use a clean new cutting surface for each sample.

Stains deposited on substrates that cannot be cut (*e.g.*, glass, metal) may be sampled with a clean new swab moistened with sterile deionized water. Use a 'sponge' technique to transfer the stain to the moistened swab; medium pressure may be required on smooth surfaces, use less pressure on rough surfaces to avoid shredding the swab batting. Swabs should be air dried in a protective environment and stored dry at room temperature, protected from light.

Swab batting can be removed from the shaft of the swab using a scalpel or scissors. The swab head can be removed from the shaft of the swab using scissors: place the swab in a microcentrifuge tube and cut the shaft as close as possible to the batting while leaving the swab head in the tube. Once cut, the shaft can be saved or discarded as per laboratory protocol.

-Supplies required: 0.6 or 1.5 ml disposable microcentrifuge tube (Certified DNase, RNase, Pyrogen, RNA/DNA Free or equivalent), filter/barrier pipette tips (low retention or equivalent), disposable transfer pipettes, Kimwipes

-Reagents required: ddH₂O and 95% EtOH

Extraction Protocol for RSID™-Semen - Positive control: A Positive Control for RSID™-Semen can be produced from 50 µl of human semen deposited on a sterile cotton swab.

Protocol

- 1.) Deposit 50 µl of human semen onto a sterile cotton swab.
 - 2.) Place swab in a 1.5 ml microcentrifuge tube using laboratory clean technique
 - 3.) Add 1 ml of RSID™-Semen Extraction Buffer and close tube.
 - 4.) Vortex tube vigorously to thoroughly wet swab.
 - 5.) Incubate at room temperature for 1-2 hours.
 - 6.) Mix 5 µl of extract with 95 µl of RSID™-Semen Running Buffer (total volume, 100 µl).
 - 7.) Load entire 100 µl into the sample window of the cassette.
 - 8.) At 10 minutes, score and record results as shown (see diagram, Technical Information Sheet, pg. 3); this will give a strong signal at the Test line.
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Extraction Protocol for RSID™-Semen - Negative control: A Negative control for RSID™-Semen can be produced from a sterile cotton swab.

Protocol

- 1.) Deposit 50 µl of sterile ddH₂O onto a sterile cotton swab.
 - 2.) Place swab in a 1.5 ml microcentrifuge tube using laboratory clean technique
 - 3.) Add 1 ml of RSID™-Semen Extraction Buffer and close tube.
 - 4.) Vortex tube vigorously to thoroughly wet swab.
 - 5.) Incubate at room temperature for 1-2 hours.
 - 6.) Mix 5 µl of extract with 95 µl of RSID™-Semen Running Buffer (total volume, 100 µl).
 - 7.) Load entire 100 µl into the sample window of the cassette.
 - 8.) At 10 minutes, score and record results as shown (see diagram, Technical Information Sheet, pg. 3); this will give no signal at the Test line.
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Extraction Protocol for RSID™-Semen from Punch or Cutting (separate from STR analysis):

Many laboratories choose to analyze evidence by testing small punches or cuttings from exhibits (swabs, fabric, etc.) before diverting the remainder of the exhibit for DNA testing. This technique can be easily integrated into RSID™-Semen testing.

Protocol

- 1.) Remove a 5 mm diameter punch or ≈20 mm² cutting from the exhibit and place in a 0.6 ml microcentrifuge tube using laboratory clean technique.

- 2.) Add 100 µl of RSID™-Semen Extraction Buffer and close tube.
 - 3.) Vortex tube vigorously to thoroughly wet punch or cutting.
 - 4.) Incubate at room temperature for 1-2 hours.
 - 5.) Briefly centrifuge extract to pellet punch or cutting.
 - 6.) Remove 20 µl of extract and bring volume to 100 µl with RSID™-Semen Running Buffer (add 80 µl of running buffer). Set a timer for 10 minutes.
 - 7.) Add extract in running buffer (100 µl) to sample well of an RSID™-Semen cassette. Start timer.
 - 8.) At 10 minutes, score and record results as shown (see diagram, Technical Information Sheet, pg. 3).
 - 9.) Due to high dose Hook effect, weak positive and negative results should be re-tested: Remove 1 µl of the original extract and bring volume to 100 µl with RSID™-Semen Running Buffer (add 99 µl of running buffer). Add extract in running buffer (100 µl) to sample well of an RSID™-Semen cassette and record result at 10 minutes.
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Extraction Protocol for RSID™-Semen from whole swab (integrated with STR analysis):

This protocol is designed to extract an exhibit swab such that *both* stain identification and DNA-STR analysis can be performed from the same sample in a single tube.

Protocol

- 1.) Place swab in 0.6 or 1.5 ml microcentrifuge tube using laboratory clean technique
- 2.) Add 200 µl of RSID™-Semen Extraction Buffer. Close tube.
- 3.) Vortex tube vigorously to thoroughly wet swab or cutting.
- 4.) Incubate at room temperature for 1-2 hours.
- 5.) Remove a 20 µl aliquot of extract for stain ID testing. Add extract to 80 µl of RSID™-Semen Running Buffer to a final volume of 100 µl in a 0.6 ml microcentrifuge tube. Set timer for 10 minutes.
- 6.) Add extract in running buffer (100 µl) to sample well of an RSID™-Semen cassette. Start timer.
- 7.) At 10 minutes, score and record results as shown (see diagram, Technical Information Sheet, pg. 3).
- 8.) Due to the high dose Hook effect, weak positive and negative results should be re-tested: Remove 1 µl of the original extract and bring volume to 100 µl with RSID™-Semen Running Buffer (add 99 µl of running buffer). Add extract in running buffer (100 µl) to sample well of an RSID™-Semen cassette and record result at 10 minutes.

- 9.) Start DNA extraction with remaining extract –
 - (a) Chelex extraction: add Chelex bead solution directly to swab, proceed as per Chelex extraction protocol.
 - (b) Phenol/Chloroform extraction: remove swab from tube and proceed with DNA extraction as per standard laboratory protocol.
 - (c) Other extraction methods: remove swab from tube and proceed with DNA extraction as per supplied protocol (e.g., Qiagen, Promega or similar).
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Validation Protocol -

This section is provided as a convenience and is meant to be a guide to documenting the validation and testing of RSID™-Semen. All laboratories must follow their own validation and testing protocols.

Validation Summary

- 1.) Record number of sample types tested. Sample types tested should include those samples commonly encountered in forensic case work.
 - 2.) Record total number of samples tested – a minimum of 5 is usually required for most laboratory audits.
 - 3.) Record test results. Include details regarding precision, sensitivity, accuracy, specificity, and reproducibility. Record the date and the initials of the analyst who performed the validation study.
 - 4.) Record approval of appropriate laboratory supervisory personnel.
 - 5.) Record date of test release. This is the date that the test passes laboratory validation and can be used for samples and casework.
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Not for in vitro diagnostic use

Manufactured by:



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