

Independent Forensics

Rapid Stain Identification Of Human Semen (RSID™-Semen) Technical Information and Protocol Sheet for Use with Universal Buffer, cat# 0230

INTENDED USE

RSID™-Semen is designed for fast, easy, and reliable detection of human semen from a variety of samples encountered by forensic laboratories including clothing, bedding, vaginal swabs, prophylactics, and stained surfaces.

The test will detect as little as 1 µL of human semen, and test results are complete within 10 minutes.

The detection protocol can be completely integrated into standard forensic laboratory procedures for DNA analysis, prior to STR analysis. The test sensitivity has been adjusted so that when semen is detected, sufficient biological material should be present to generate an STR profile (exceptions include low sperm count semen or semen from vasectomized men).

This is the first commercially available confirmatory test for human semen. No other human body fluids or animal semen samples tested cross react with RSID™-Semen. The immunochromatographic strip test uses dual monoclonal antibodies specific for human semenogelin: *the test does not detect PSA or P30*.

The new RSID™ - Universal Buffer is designed for use with Independent Forensics' RSID™-Saliva, RSID™-Semen and RSID™-Blood tests. Using RSID™ - Universal Buffer, forensic labs can now extract one sample using a single buffer, and test for three different body fluids: *one sample, one buffer, three body fluid tests*. The use of a single buffer will enable forensic laboratories to minimize sample consumption without compromising the specificity or sensitivity for the detection of saliva, semen, and blood.

Introduction

RSID™-Semen is a lateral flow immunochromatographic strip test that uses two anti-human semenogelin monoclonal antibodies in a lateral flow format, to detect the presence of semenogelin. Semenogelin is a protein produced by the seminal vesicles and is responsible for the coagulum associated with ejaculate. RSID™-Semen uses.

RSID™-Semen is specific for human semen and has numerous advantages over other methods for semen detection, including increased sensitivity, specificity, and speed. Current identification methods for semen are presumptive (provide a basis for continued analysis of the tested exhibit, but are not specific for semen), and are therefore open to legal and scientific challenge.

Principle of the Test

RSID™-Semen is an immunochromatographic assay that uses two mouse monoclonal antibodies specific for human semenogelin. One of these antibodies is conjugated to colloidal gold and is deposited on a conjugate pad beneath the sample window. The other antibody is striped onto the

“Test line” on a membrane attached to the conjugate pad. The “Control line” on the membrane consists of anti-mouse IgG antibody and is used as an internal control.

Following the addition of test liquid to the conjugate pad, sample and antibodies (complexed and free) are transported by bulk fluid flow to the membrane. The immobilized anti-semenogelin antibodies on the test line capture the semenogelin antigen-antibody-colloidal gold complexes, producing a red line at the Test position. If no human semenogelin is present in the sample, no red line will appear. A red line should appear at the Control position on each strip. This demonstrates that the sample fluid was transported through the length of the test, and that the components of the strip test are working correctly.

Principle of the RSID™ - Universal Buffer

The components of the RSID™ - Universal Buffer are designed to efficiently extract semenogelin from questioned stains and swabs and to dissolve the antibody-colloidal gold conjugate from the conjugate pad, maintain an extract at the appropriate pH, and facilitate correct running of the test. The components of the Universal Buffer have been optimized to allow *one sample, one buffer, three body fluid tests* without compromising sensitivity or specificity of the analysis. Components include buffer and salts (Tris, NaCl, KCl) for physiological stability, a chelating agent (EDTA) for stability, detergents and surfactants (Triton X-100 and Tween 20) for extraction efficiency and solubility maintenance, protein (BSA) for reducing non-specific adsorption and loss, and a preservative (sodium azide).

Reagents and Materials Provided

- i) Test cassettes: 25 cassettes individually wrapped and sealed in a moisture-proof foil (a silica gel desiccant pouch has been added for increased shelf life.)
- ii) 30 mL of RSID™- Universal Buffer.

Protocol for Positive Control

Positive controls for RSID™-Semen can be produced from 50 µL of human semen deposited on a cotton swab. The semen swab should be extracted in 1 mL of RSID™- Universal Buffer for 1-2 hours at room temperature; 5 µL of this extract should be diluted in 95 µL of RSID™- Universal Buffer (total volume 100 µL). Load all 100 µL into the sample well; this will give a robust positive signal.

Protocol for Negative control

A negative control for RSID™-Semen can be produced from extracting a sterile cotton swab in the same manner as your samples. Alternatively, 100 µL of RSID™- Universal Buffer may be added to the cassette and run as normal.

Suggested Extraction Protocol for Sample Analysis

Forensic samples obtained on cotton swabs should be extracted in 200-300 μL of RSID™- Universal Buffer for 1-2 hours. Alternatively, a portion of a swab may be used, and sufficient RSID™- Universal Buffer should be added to easily cover the sample. Stains on fabric or paper should be sampled by taking a punch or cutting ($\approx 20 \text{ mm}^2$) of the item. The punch or cutting should be extracted in 100 μL of RSID™- Universal Buffer for 1-2 hours. A general guideline of a maximum of 10% of extract, up to a maximum of 20 μL should be run. The remainder of the extract can be processed for STR analysis using any one of a number of DNA extraction protocols. The buffer provided is STR free and contains a DNA stabilizer. The provided buffers do not interfere with extraction or amplification.

Strip Test Assay Procedure

Note: Assays should be performed at room temperature. It is recommended that a positive and negative control be included with every assay.

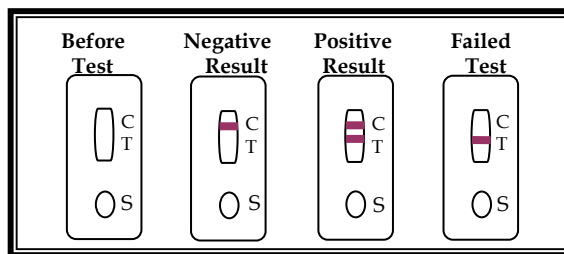
1. Remove cassette from the foil pouch. Discard silica gel desiccant.
2. Combine extract aliquot (max of 20 μL) with RSID™- Universal Buffer to bring test sample to a total volume of 100 μL .
3. Add sample in RSID™- Universal Buffer to sample window. Start timing at the point the sample is added to the sample window.
4. Due to the High Dose Hook Effect, samples giving a weak positive or negative result should be diluted 1:20 and re-tested. For example: If 20 μL from a 200 μL swab extract gives a weak positive or negative result, 1 μL from the original extract should be added to 99 μL RSID™- Universal Buffer and analyzed on a new cassette (see High Dose Hook Effect below for details).
5. At 10 minutes, score and record results as shown in the Scoring Results diagram shown below.

Alternatively, users may add 100 μL from the extraction to the cassette of RSID™-Semen. This will have little to no effect on the sensitivity or specificity of the test; however, any problems encountered by the high dose hook effect or using a concentrated sample (e.g., altered pH) may be avoided if the extraction is diluted in RSID™- Universal Buffer, as described above.

Scoring Results

RSID™-Semen should be evaluated *exactly* 10 minutes after the addition of sample. Fig. 1 illustrates expected results:

- i) A visible red line at the Control (C) position only indicates a negative result.
No human semen detected.
- ii) Visible red lines at both the Control (C) and Test (T) positions indicate a positive result.
Human semen detected.
- iii) A visible red line at the Test (T) position only indicates a failed test.
Test failure, no conclusion possible.



Stability and Storage

RSID™-Semen cassettes should be stored at room temperature. RSID™- Universal Buffer should be stored at 2-8°C. Do not use buffer or cassettes after the printed expiration date.

Specificity

RSID™-Semen is specific for human semenogelin. No cross-reactivity with human saliva, whole blood, vaginal fluid, menstrual blood, breast milk or urine has been observed.

No cross reactivity with animal semen has been observed. Species tested: chimp, gorilla, dog, cat, mouse, cow, horse, pig, goat, and sheep.

Test Sensitivity

The detection limit for RSID™-Semen, used as suggested, is $< 1 \mu\text{L}$ of human semen.

Undiluted semen should *not* be used with RSID™-Semen, as the viscosity of the sample prevents proper release of the conjugate from the conjugate pad. The tested sample should first be deposited on a sterile cotton swab, extracted in RSID™- Universal Buffer, and diluted as needed in RSID™- Universal Buffer before analysis with RSID™-Semen.

High Dose Hook Effect

A high dose Hook effect refers to reduction on test line signal on immunochromatographic strip tests when very high levels of target are present in the tested sample. Under these conditions, unbound target antigen can reach the test line *before* the colloidal gold-labeled antibody-bound antigen, occupying the test line antibody sites and possibly resulting in a weak positive or false negative result.

Weak positives can be observed with RSID™-Semen when samples containing large amounts of human semen (≈ 3 to 50 μL) are analyzed. 20-fold dilution of these samples and re-testing with RSID™-Semen eliminates the weak positive and false negative results (see Validation Summary online).

Not for in vitro diagnostic use
Manufactured by:



Independent Forensics

4600 ROOSEVELT RD., STE. 201 - HILLSIDE, IL 60162

TEL. 866-434-2400 FAX 708-978-5115

WWW.IFI-TEST.COM/RSID.HTML