## Independent Forensics

# Rapid Stain Identification of Human Semen (RSID™-Semen)

### Technical Information and Protocol Sheet for Use with Dual Buffer System Reduced Incubation Time, cat# 0200

### 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.

Based on validation studies using positive control swabs made with 50 µL semen, the test will detect as little as 10 nL of human semen, and test results are complete within 10 minutes.

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.***

### Introduction

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

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.

### 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) 5 mL of RSID™-Semen Running Buffer
iii) 25 mL of RSID™-Semen Extraction Buffer

To determine if RSID™-Semen is compatible with shorter sample extraction times, a series of time course experiments were undertaken with control swabs, aged samples (several years old), trace semen samples, and semen on fabrics. These data clearly demonstrated that similar results could be obtained from all tested sample types using incubation times as short as 10 seconds to as long as 1 hour (to view the data, go to www.ifi-test.com/rsidtm-documentation**). For shorter extraction times (i.e. 10 seconds to 1 minute) the sample must be shaken during the incubation for optimal extraction of semenogelin.** Longer incubation times (*i.e.,* 5-60 minutes) are optional.

### 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™-Semen Extraction Buffer for 10 seconds, while shaking or longer, at room temperature; 5 µL of this extract should be diluted in 95 µL of RSID™-Semen Running Buffer (total volume 100 µL). Load all 100 µL into the sample well; this will give a clear 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, 20 µL of Extraction Buffer may be added to 80 µL of Running Buffer and run as normal.

### Suggested Extraction Protocol for Sample Analysis with New Shorter Incubation time

Forensic samples obtained on cotton swabs should be extracted in 300-400 µL of RSID™-Semen Extraction Buffer: **shake for 10 seconds**, longer incubation times are optional. We have obtained similar results with incubation times ranging from 10 seconds to an hour. Alternatively, a portion of a swab may be used, and sufficient RSID™-Semen Extraction Buffer should be added to easily cover the sample. Stains on fabric or paper should be sampled by taking a punch or cutting (≈ 20 $mm^2$) of the item. The punch or cutting should be extracted in 100 µL of RSID™-Semen Extraction Buffer for 10 seconds, or longer. 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 positive and negative controls 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™-Semen Running Buffer to bring test sample to a total volume of 100 µL
3. Add sample in RSID™-Semen Running 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 1:10 and re-tested. For example: If 10 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™ Semen Running 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.

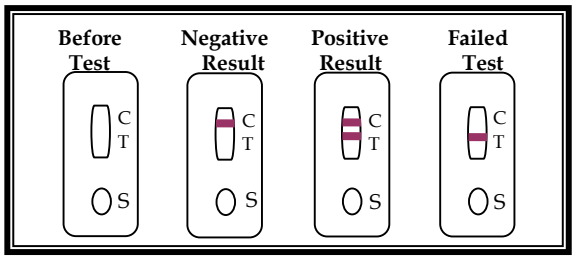
## Scoring Results

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™-Semen Extraction and Running Buffers should be stored at 2-8°C. Do not use buffers 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 10 nl of human semen. This detection limit is based on testing dilutions from extracts of positive control swabs made with 50 µL 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™-Semen Extraction Buffer, and diluted as needed in RSID™-Semen Running Buffer before analysis with RSID™-Semen.

## High Dose Hook Effect

A high dose Hook effect refers to weak positive or false negative results seen with 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 resulting in a weak positive or false negative result.

We have observed weak positives and false negatives with RSID™-Semen when samples containing large amounts of human semen (≈ 3 to 50 µl) were analyzed. 20-fold dilution of these samples and re-testing with RSID™-Semen eliminated the weak positive and false negative results (see Validation Summary online).

***User Note***: Under standard laboratory testing, users of RSID™-Semen may observe weak positive or false negative results due to the High Dose Hook Effect. Therefore, any weak positive or negative result from RSID™-Semen should be confirmed by diluting the sample 1:20 and re-testing. If re-testing of the diluted sample results in a stronger positive signal, the original result was caused by the high dose Hook effect and a large amount of semen is present in the sample. If re-testing of the diluted sample is once again weakly positive or negative, the original result is confirmed.

*Not for in vitro diagnostic use*

*Manufactured by*:

Independent Forensics

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