

Independent Forensics  
RSID™ - Universal buffer  
**Technical Information and Protocol Sheet**

### INTENDED USE

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.

### Principle of the RSID™ Test

The three Rapid Stain Identification (RSID™) products (RSID™-Saliva, RSID™-Semen and RSID™-Blood) are lateral flow immunochromatographic strip tests designed to detect the presence of saliva, semen, or blood. Each lateral flow assay uses two mouse monoclonal antibodies specific for  $\alpha$ -amylase, semenogelin, or glycophorin A. For each test, one of the body fluid specific antibodies (Ab1) is conjugated to colloidal gold and is deposited on a conjugate pad beneath the sample window. The other antibody (Ab2) 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 positive control.

Following the addition of test liquid to the conjugate pad, sample and antibodies (Ab1, complexed and free) are transported by bulk fluid flow to the membrane. The immobilized Ab2 antibodies on the test line capture the antigen-antibody-colloidal gold complexes, producing a red line at the Test position. If no antigen 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 membrane, and that the components of the test are working correctly.

### Principle of the Universal Buffer

The original formulation of the RSID™ laboratory kits contained a dual buffer system: an extraction buffer and a running buffer specific for each kit; six buffers in all. RSID™-Saliva, RSID™-Semen and RSID™-Blood extraction buffers were designed to efficiently extract the specific antigen ( $\alpha$ -amylase, semenogelin, and glycophorin A, respectively) from questioned stains and swabs. RSID™-Saliva, RSID™-Semen, and RSID™-Blood running buffers were designed 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. Components of the extraction and running buffers 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).

The components of the RSID™- Universal Buffer are similar to the components of the original extraction and running buffers of the RSID™-Saliva, RSID™-Semen, and RSID™-Blood laboratory kits. However, the components have been optimized to allow *one sample, one buffer, three body fluid tests* without compromising sensitivity or specificity of the analysis.

### Reagents and Materials Provided

i) 30 ml of RSID™-Universal Buffer

### Protocol for Positive Control

Positive controls for RSID™-Saliva, RSID™-Semen and RSID™-Blood can be produced from 50  $\mu$ L of human saliva, semen, or blood, deposited on a cotton swab and air-dried. The swab should be extracted in 1 ml of RSID™- Universal Buffer for 1-2 hours at room temperature; 5  $\mu$ L of this extract should be diluted in 95  $\mu$ L of RSID™- Universal Buffer (total volume 100  $\mu$ L). Load all 100  $\mu$ L into the sample well; this will give a robust positive signal on RSID™-Saliva, RSID™-Semen, and RSID™-Blood cassettes.

### Protocol for Negative control

A blank cotton swab can be extracted with 1 mL of RSID™- Universal Buffer and 100  $\mu$ L of RSID™- Universal Buffer may be added to the cassette as a negative control.

### Suggested Extraction Protocol for Sample Analysis

Forensic samples obtained on cotton swabs should be extracted in 200-300  $\mu$ L 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 ( $\approx 20$  mm<sup>2</sup>) of the item. The punch or cutting should be extracted in 100  $\mu$ L of RSID™- Universal Buffer for 1-2 hours. A general guideline of a maximum of 10% of extract, up to a maximum of 20  $\mu$ L should be tested. The remainder of the extract can be processed for STR analysis using any one of a number of DNA extraction protocols. The RSID™- Universal Buffer is STR free and contains a DNA stabilizer and therefore, will 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.

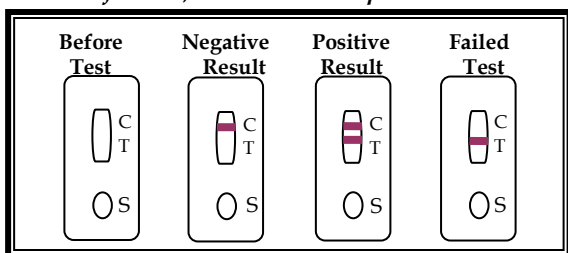
2. Combine extract aliquot (max of 20 µL) with RSID™- Universal Buffer to bring test sample to a total volume of 100 µL.
3. Add sample in RSID™- Universal Buffer to sample window. Start timing at the point the sample is added to the sample window.
4. Due to the High Dose Hook Effect when using RSID™- Semen, 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, the user may add 100 µL from the extraction straight to the cassette of RSID™-Saliva, RSID™-Semen and RSID™-Blood. This will have little to no effect on the sensitivity or specificity of RSID™-Saliva or RSID™-Blood; however, the high dose hook effect of RSID™-Semen may be more pronounced if the sample is not diluted. In addition, any problems encountered with a concentrated sample (*i.e.* altered pH) may be avoided if the extraction is diluted in RSID™- Universal Buffer, as described above.

## Scoring Results

RSID™ products 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 body fluid detected.**
- ii) Visible red lines at both the Control (C) and Test (T) positions indicate a positive result.  
**Body fluid 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™- Universal Buffer should be stored at 2-8°C. Do not use buffer after the printed expiration date.

## Test Sensitivity

The detection limit for RSID™-Saliva, RSID™-Semen and RSID™-Blood, used as suggested, is still < 1 µL of human body fluid, identical to the reported limit of detection using the original dual buffer system. The limit of detection of RSID™-Blood is slightly increased when testing aged samples due to less efficient extraction from the substrate.

Undiluted body fluids should **not** be used with any RSID™ products, 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, air-dried, extracted in RSID™- Universal Buffer, and diluted as needed in RSID™- Universal Buffer before analysis with RSID™ tests.

## High Dose Hook Effect

A high dose hook effect refers to the decrease in test signal that can occur on immunochromatographic strip tests when very high levels of target are present in the tested sample. This decrease in signal intensity can result in a false negative result. 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 (≈ 10 to 50 µL) are 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.

## Suggested Validation Protocol

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

- 1) Record 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.
- 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.



Not for *in vitro* diagnostic use

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