

**Independent Forensics**  
**Rapid Stain Identification of Human Saliva (RSID™-Saliva)**  
**Technical Information & Protocol Sheet for Use with Universal Buffer,**  
**Reduced Incubation Time Cat# 0130**

**INTENDED USE**

RSID™-Saliva is designed for fast, easy, and reliable detection of human saliva from a variety of samples encountered by forensic laboratories including envelopes, glass bottles, aluminum cans, plastic lids and swabs of possibly contaminated surfaces.

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

RSID™-Saliva is an immunochromatographic strip test with dual monoclonal antibodies specific for human salivary  $\alpha$ -amylase. No cross-reaction has been observed with blood, semen, urine, vaginal secretions, or menstrual blood. Low level detection of breast milk and human fecal samples are observed: reactivity of saliva is  $\approx$ 40 times stronger than breast milk (see Validation Study for details).

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.

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**Introduction**

RSID™-Saliva is a lateral flow immunochromatographic strip test designed to detect the presence of human salivary  $\alpha$ -amylase, an enzyme found in human saliva; the enzyme's physiological role is to aid in the digestion of dietary starches.

RSID™-Saliva is specific and has numerous advantages over current enzymatic methods for amylase detection, including increased sensitivity, specificity, and speed. Current enzyme activity-based methods for saliva detection are not specific for human saliva and cross react with bacterial, fungal and pancreatic  $\alpha$ -amylase, which all score positive when enzymatic assays for amylase activity are used for saliva detection.

RSID™ - Saliva uses two anti-human salivary amylase, monoclonal antibodies, in a lateral flow format, that detects the *presence* of salivary amylase, rather than the *activity* of the enzyme (see Specificity below).

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**Principle of the Test**

RSID™ - Saliva uses two mouse monoclonal antibodies specific for human salivary  $\alpha$ -amylase. 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. Attached to the

other end of the membrane is the wick, which absorbs the tested fluid and running buffer at the completion of the test thus preventing back-flow of the sample. Once the tested fluid is added to the sample window, the running buffer and sample diffuse through the conjugate pad, re-dissolving the gold-conjugated detection antibodies. If human salivary amylase is present in the sample an antigen-colloidal gold conjugated antibody complex will form. Sample and antibodies (complexed and free) are transported by bulk fluid flow to the membrane section of the strip test. The immobilized anti- $\alpha$ -amylase antibodies on the test line capture the amylase-antibody-gold complexes, producing a red line at the Test position. If no human salivary amylase is present in the sample, then gold-conjugated antibody-antigen complexes cannot form, and colloidal gold will not be accumulated at the Test line. 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.

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**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

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To determine if RSID™-Saliva is compatible with shorter sample extraction times, a series of time course experiments were undertaken with control swabs, aged samples (several years old), trace saliva samples, and saliva 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](http://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  $\alpha$ -amylase.** Longer incubation times (i.e., 5-60 minutes) are optional.

**Protocol for Positive Control**

Positive controls for RSID™-Saliva can be produced from 50 µL of human saliva deposited on a cotton swab. The saliva swab should be extracted in 1 mL of RSID™-Universal Buffer for 10 seconds while shaking, or longer, at room temperature; 20 µL of this extract should be diluted in 80 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™-Saliva can be produced from extracting a sterile cotton swab in the same manner as

your samples. Alternatively, 100  $\mu\text{L}$  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 300-400  $\mu\text{L}$  of RSID™-Universal Buffer: **shake for 10 seconds**, longer incubation times are optional. 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  $\mu\text{L}$  of RSID™-Universal Buffer for 10 seconds or longer. A general guideline of a maximum of 10% of extract, up to a maximum of 20  $\mu\text{L}$  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.

### 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.

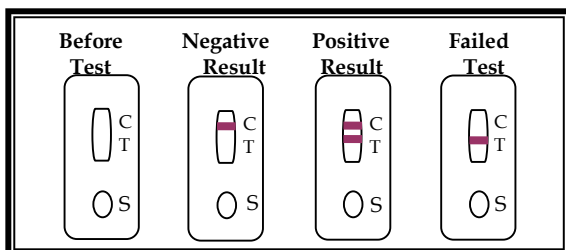
1. Remove cassette from the foil pouch. Discard silica gel desiccant.
2. Combine extract aliquot (max of 20  $\mu\text{L}$ ) with RSID™-Universal Buffer to bring test sample to a total volume of 100  $\mu\text{L}$ .
3. Add sample in RSID™- Universal Buffer to sample window. Start timing.
4. At 10 minutes, score and record results as shown in the Scoring Results diagram shown below.

Alternatively, users may add 100  $\mu\text{L}$  from the extraction to the cassette of RSID™-Saliva. This will have little to no effect on the sensitivity or specificity of the test; however, any problems encountered by 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™-Saliva 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 alpha-amylase detected.*
- ii) Visible red lines at both the Control (C) and Test (T) positions indicate a positive result.  
*Alpha-amylase 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™-Saliva 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

The RSID™-Saliva test is specific for human salivary  $\alpha$ -amylase. No cross-reaction has been observed with blood, semen, urine, vaginal secretions, or menstrual blood. Low level detection of breast milk and human fecal samples are observed: reactivity of saliva is  $\approx 40$  times stronger than breast milk (see validation study for details).

No cross reactivity has been observed with saliva from the following animals and pets: dog, opossum, guinea pig, woodchuck, cow, domestic cat, domestic rabbit, tokay gecko, cuckoo, mongoose, chameleon, domestic pig, llama, sheep, horse, goat, grey gull, ferret, hedgehog, skunk, lion, tiger, rhinoceros, marsh snake, Sykes monkey, Capuchin monkey, tamarin, and marmoset. A positive signal was obtained from the saliva of gorilla.

### Test Sensitivity

The detection limit for RSID™-Saliva, used as suggested is 10 nL of human saliva. This detection limit is based on testing dilutions from extracts of positive control swabs made with 50  $\mu\text{L}$  saliva.

Undiluted saliva should *not* be used with RSID™-Saliva, 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 the RSID™-Saliva test kit.

### High Dose Hook Effect

A *high dose Hook effect* refers to the decrease in test line intensity seen with immunochromatographic strip tests when very high levels of target are present in the tested sample. Under these conditions, unbound salivary  $\alpha$ -amylase antigen can reach the test line *before* the colloidal gold-labeled antibody-bound antigen, potentially resulting in a false negative result.

We have tested RSID™-Saliva with human saliva extracts containing up to 50  $\mu\text{L}$  of human saliva (i.e., 50  $\mu\text{L}$  of saliva on a cotton swab, extracted with 100  $\mu\text{L}$  RSID™ Extraction Buffer, and the entire extract added to the sample window) with no false negative results. Under standard laboratory testing, users will not observe false negative results due to high dose Hook effects.

Not for *in vitro* diagnostic use  
Manufactured by:



**Independent Forensics**

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