Independent Forensics

Rapid Stain Identification of Human Saliva (RSID™-Saliva)

Technical Information & Protocol Sheet for Use with Dual Buffer System, Cat# 0100

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.

The test will detect as little as 1 µL of human saliva. Test results are complete within 10 minutes.

The detection protocol can be completely integrated into standard forensic laboratory procedures for DNA-STR analysis. The test sensitivity has been adjusted so that when saliva is detected, sufficient biological material should be present to generate an STR profile.

RSID™-Saliva is an immunochromatographic strip test with dual monoclonal antibodies specific for human salivary α-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 ≈40 times stronger than breast milk (see Validation Study for details).

Introduction

RSID™-Saliva is a lateral flow immunochromatographic strip test designed to detect the presence of human salivary α-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 α-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).

Principle of the Test

RSID™-Saliva uses two mouse monoclonal antibodies specific for human salivary α-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. The "Control line" on the membrane consists of anti-mouse IgG antibody and is used as a positive control. 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-α-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. The anti-mouse IgG on the control line captures any mouse antibodies flowing past the test line, producing a red line at the Control position and ensuring that the sample fluid was transported through the length of the test and that the components of the strip test are working correctly.

RSID™ -Saliva, IFI Cat # 0100, laboratory kit contains a dual buffer system: an extraction buffer and a running buffer specific for RSID™-Saliva. RSID™-Saliva extraction buffer is designed to efficiently extract α-amylase from questioned stains and swabs. RSID™-Saliva running buffer is 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).

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™-Saliva Running Buffer
iii) 25 mL of RSID™-Saliva Extraction Buffer

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™-Saliva Extraction Buffer for 1-2 hours at room temperature; 20 µL of this extract should be diluted in 80 µL of RSID™-Saliva Running 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, 20 µL of Extraction Buffer may be added to 80 µL of Running Buffer 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™-Saliva Extraction Buffer for 1-2 hours. Alternatively, a portion of a swab may be used, and sufficient RSID™-Saliva 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™-Saliva Extraction 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™-Saliva Running Buffer to bring test sample to a total volume of 100 µL.
3. Add sample in RSID™-Saliva Running Buffer to sample window. Start timing at the point the sample is added to the sample window.
4. At 10 minutes, score and record results as shown in the Scoring Results diagram shown below.

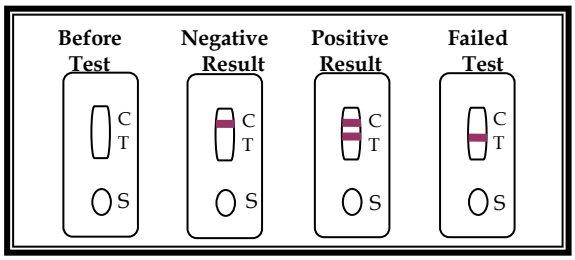
Scoring Results

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™-Saliva Extraction and Running Buffers should be stored at 2-8°C. Do not use buffers or cassettes after the printed expiration date.

Specificity

The RSID™-Saliva test is specific for human salivary α-amylase. No cross-reaction has been observed with blood, semen, urine, vaginal secretions, or menstrual blood. Low level detection of human breast milk and human fecal samples are observed: reactivity of saliva is ≈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 <1 µL of human 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™-Saliva Extraction Buffer, and diluted as needed in RSID™-Saliva Running 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 signal intensity on immunochromatographic strip tests when very high levels of target are present in the tested sample. Under these conditions, unbound salivary α-amylase antigen could 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 µL of human saliva (i.e., 50 µL of saliva on a cotton swab, extracted with 100 µL 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
500 Waters Edge, Suite 210, Lombard IL 60148
p 866.434.2400, f 708.978.5115
WWW.IFI-TEST.COM/RSID