

Independent Forensics
Rapid Stain Identification of Human Blood (RSID™ -Blood)
Technical Information and Protocol Sheet for Universal Buffer, cat# 0330

INTENDED USE

RSID™-Blood is designed for fast, easy, and reliable detection of human blood from a variety of samples encountered by forensic laboratories including fabric and stained surfaces.

The test can detect as little as 1 µL of human blood, and the strip test results are complete within 10 minutes.

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

This is the first commercially available confirmatory test for human blood. No other human body fluids or animal blood samples tested (including ferret, skunk, and primate) cross react with RSID™-Blood (see Specificity for fluids and species tested). Also, unlike other commercially available blood detection strip tests, RSID™-Blood does not exhibit a high dose Hook effect. The RSID™-Blood immunochromatographic strip test uses dual monoclonal antibodies specific for human glycophorin A, *not* hemoglobin.

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

Rapid Stain Identification of Human Blood (RSID™-Blood) uses two monoclonal antibodies in a lateral flow format to detect the presence of human glycophorin A. Glycophorin A is abundantly and specifically expressed in red blood cells where it is thought to prevent cellular aggregation.

RSID™-Blood is specific for human blood and has numerous advantages over other methods of blood detection, including increased specificity, and no High Dose Hook effect. Current identification methods for blood are presumptive (provide a basis for continued analysis of the tested exhibit, but are not specific for human blood), and are therefore open to legal and scientific challenge.

Principle of the Test

RSID™-Blood is an immunochromatographic assay that uses two mouse monoclonal antibodies specific for human glycophorin A. 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” of 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.

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 antibodies. If human glycophorin A is present in the sample, an antigen-antibody-colloidal gold complex will form. Sample and antibodies (complexed and free) are transported by bulk fluid flow to the membrane phase of the strip test. The immobilized anti-glycophorin A antibodies on the test line capture the glycophorin A antigen-antibody-colloidal gold complexes, producing a red line at the Test position. If no human glycophorin A is present in the sample, then gold-conjugated antibody-antigen complexes do not form, and colloidal gold will not be accumulated at the Test line. The anti-mouse IgG on the control line captures mouse antibodies flowing past the test line, producing a red line at the Control position. 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 glycophorin A 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™-Blood can be produced from 50 µL of human blood deposited on a cotton swab. The blood swab should be extracted in 1 mL of RSID™- Universal Buffer for 1-2 hours 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™-Blood 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 cutting 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

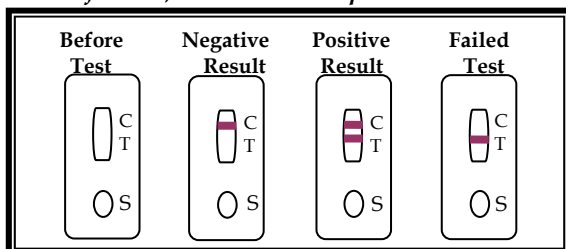
Note: Assays should be performed at room temperature. It is recommended that a positive and negative control be included with every assay.

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. At 10 minutes, score and record results as shown in the Scoring Results diagram shown below.

Scoring Results

RSID™-Blood 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 Glycophorin A detected.
- ii) Visible red lines at both the Control (C) and Test (T) positions indicate a positive result.
Glycophorin A 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™-Blood cassettes should be stored at room temperature. RSID™- Universal Buffer should be stored at 2-8°C. Do not use buffers or cassettes after the printed expiration date.

Specificity

RSID™-Blood is specific for human glycophorin A. No cross-reactivity with human saliva, semen, breast milk, amniotic fluid, vaginal fluid or urine has been observed.

No cross reactivity with animal blood has been observed. Species tested: ferret, skunk, opossum, dog, cat, cow, pig, chicken, owl, horse, goat, turtle, elk, deer, tiger, alpaca, orangutan, gorilla, spider monkey, bonobo, and baboon.

Test Sensitivity

The sensitivity for RSID™-Blood, used as suggested, is less than $< 1 \mu\text{L}$ of human blood. When using RSID™- Universal Buffer, the limit of detection (the minimal volume of blood required for a positive signal) is slightly increased from $\sim 50 \text{ nL}$ to $\sim 100 \text{ nL}$ blood.

Undiluted blood should *not* be used with RSID™-Blood, 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™-Blood.

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 antigen are present in the tested sample. Under these conditions, unbound bio-marker antigen can reach the test line *before* the colloidal gold-labeled antibody-bound bio-marker antigen, resulting in a false negative result.

We have tested RSID™-Blood with human blood extracts containing up to 20 μL of human blood (i.e., 100 μL of blood on a cotton swab, extracted with 500 μL RSID™- Universal Buffer, and 100 μL of extract added to the sample window) with no false negative results. Under standard laboratory testing and relevant blood concentration ranges, users will not observe false negative results due to the high dose Hook effect.

Not for in vitro diagnostic use



Manufactured by:

Independent Forensics

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

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

WWW.IFI-TEST.COM/RSID.HTML