

Independent Forensics  
Rapid Stain Identification  
Of Human Blood (RSID™-Blood)  
Provided Protocols

### GENERAL GUIDELINES

When possible, stains deposited on fabric or other substrates that can be easily cut, should be dissected to preserve at least half of the questioned stain in order to allow for retesting. Scissors or scalpels used to cut the underlying substrate of questioned stains should be washed in 95% ethanol and deionized water, and then dried with a fresh Kimwipe before use, and between each cutting, with special emphasis on the hinge region of scissors. We recommend immersing scissors and scalpels sequentially in 95% ethanol and deionized water between uses. Use a clean new cutting surface for each sample.

Stains deposited on substrates that cannot be cut (e.g., glass, metal) may be sampled with a clean new swab moistened with sterile deionized water. Use a 'sponge' technique to transfer the stain to the moistened swab; medium pressure may be required on smooth surfaces, use less pressure on rough surfaces to avoid shredding the swab batting. Swabs should be air-dried in a protective environment and stored dry at room temperature, protected from light.

Swab batting can be removed from the shaft of the swab using a scalpel or scissors. The swab head can be removed from the shaft of the swab using scissors: place the swab in a microcentrifuge tube and cut the shaft as close as possible to the batting while leaving the swab head in the tube. Once cut, the shaft can be saved or discarded as per laboratory protocol.

-Supplies required: 0.6 or 1.5 ml disposable microcentrifuge tube (Certified DNase, RNase, Pyrogen, RNA/DNA Free or equivalent), filter/barrier pipette tips (low retention or equivalent), disposable transfer pipettes, Kimwipes

-Reagents required: ddH<sub>2</sub>O and 95% EtOH

### Extraction Protocol - Positive control for Stain ID

A positive Control for RSID™-Blood can be produced from 50 µl of human blood deposited on a sterile cotton swab.

#### Protocol

- 1.) Apply 50 µl of human blood to a sterile cotton swab.
- 2.) Cut swab head into a 1.5 ml microcentrifuge tube, add 1 ml of RSID™-Blood Extraction Buffer, vortex tube and incubate at room temperature for 1-2 hours.
- 3.) Mix 20 µl of blood extract with 80 µl of RSID™-Blood Running Buffer (total volume, 100 µl).
- 4.) Load entire 100 µl into the sample window of the cassette.
- 5.) Record result at 10 minutes. This will produce a strong positive result.

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### Extraction Protocol - Negative control for Stain ID

A negative control for RSID™-Blood can be produced from a sterile cotton swab.

#### Protocol

- 1.) Apply 50 µl of ddH<sub>2</sub>O to a sterile cotton swab.
- 2.) Cut swab head into a 1.5 ml microcentrifuge tube, add 1 ml of RSID™-Blood Extraction Buffer, vortex tube and incubate at room temperature for 1-2 hours.
- 3.) Mix 20 µl of extract with 80 µl of RSID™-Blood Running Buffer (total volume, 100 µl).
- 4.) Load entire 100 µl into the sample window of the cassette.
- 5.) Record result at 10 minutes. This will produce a negative result.

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### Extraction Protocol, Single Tube - Stain ID Integrated into STR Analysis

This protocol is designed to extract a probative swab such that *both* stain identification and DNA-STR analysis can be performed from the same sample in a single tube format.

### Protocol

1.) Cutting: Place cutting in 0.6 or 1.5 ml microcentrifuge tube using laboratory clean technique.

Swab: Place swab head or batting into 0.6 or 1.5 ml microcentrifuge tube using laboratory clean technique.

2.) Add 200-300 µl of RSID™-Blood Extraction Buffer (0.6 ml or 1.5 ml tube). Close tube.

3.) Vortex tube vigorously to thoroughly wet swab or cutting.

4.) Incubate at room temperature for 1-2 hours.

5.) Remove a 20 to 40 µl aliquot of extract for stain ID testing. Add extract to either 80 or 60 µl of RSID™-Blood Running Buffer (final volume of 100 µl) in a 0.6 ml microcentrifuge tube. Set timer for 10 minutes.

6.) Add extract in RSID™-Blood Running Buffer (100 µl) to sample well of an RSID™-Blood cassette. Start timer.

7.) At 10 minutes, score and record results as shown (see diagram, Technical Information Sheet, pg. 3).

8.) Start DNA extraction with remaining extract -  
(a) Chelex extraction: add Chelex bead solution directly to swab or cutting, proceed as per Chelex extraction protocol.  
(b) Phenol/Chloroform extraction: remove cutting or swab from tube. Proceed with DNA extraction as per standard laboratory protocol.  
(c) Other extraction methods: remove cutting or swab from tube and proceed with DNA extraction as per supplied protocol (e.g., Qiagen, Promega or similar).

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### **Extraction Protocol, Multiple Tube Method - Stain ID Distinct from STR Analysis**

Many laboratories choose to analyze evidence swabs by testing small cuttings from the swab batting before diverting the remainder of the swab for DNA testing. This technique can be easily integrated into RSID™-Blood testing.

### Protocol

1.) Remove cutting(s) from the swab batting and place into a 0.6 ml microcentrifuge tube.

2.) Add 50 µl of RSID™-Blood Extraction Buffer, vortex thoroughly, and incubate for 1 hour at room temperature.

3.) Briefly centrifuge extract to pellet cutting.

4.) Transfer all liquid (~40 µl) to a fresh tube and bring volume to 100 µl with RSID™-Blood Running Buffer (add ~60 µl of running buffer). Set a timer for 10 minutes.

5.) Add extract in running buffer (100 µl) to sample well of an RSID™-Blood cassette. Start timer.

6.) Record result at 10 minutes.

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### **Validation Protocol -**

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

### Validation Summary

1.) Record number of 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. Record the date and the initials of the analyst who performed the validation study.

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.

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