MBLF004 Insta-LF™ Rapid-B Kit

Kit Content:

Product Code	Material Provided	20 Tests
DS1402	Human Blood Detection-Test Cassette with desiccant in individual foil pouch	20 nos
DS1403	Filled Extraction Buffer Tubes	20 nos
DS1404	Extra-extraction Buffer	2 ml
PW005	Sterile Cotton Swab	20 nos

Introduction: Insta-LF™ Rapid-B test serves the rapid identification of human blood for forensic purpose. The identification is based on the detection of cell membrane antigen of erythrocyte in the sample by a specific antigen-antibody reaction. The result is interpreted visually by the appearance of the test line and control line in human blood positive samples. The test is easy to perform and can be performed directly on site.

Most human blood detection tests that are currently available in the market for forensic use, target hemoglobin protein for detection.

CONCERN:

- Hemoglobin targeted lateral flow assay shows cross reaction with few animal bloods, also it shows cross reactivity with other human body fluids
- Furthermore, hemoglobin targeted lateral flow tests show high-dose hook effect which can results in false negative results when analyte is present in high concentration
- For the precise results, hemoglobin based lateral flow test needs sample dilution and no proper guidelines are given on dilution factor

SOLUTION:

- To suffice the need of superior blood detection kit, HiMedia has developed erythrocyte cell membrane antigen-based
★ detection lateral flow test using specificity and sensitivity enhancing conjugation and buffer technology for human blood identification
- The HiMedia's lateral flow test shows no cross reactivity with animal blood nor with other human body fluids and
★ also not susceptible to high-dose hook effect which causes false negative results

Principle of the test:

This test provides a lateral flow immunoassay for *in-vitro* objective identification of presence or absence of cell membrane antigen of erythrocyte in the sample. This technique uses an antigen-antibody interaction that is very specific.

A pair of antibodies against human cell membrane antigen of erythrocyte are used in this assay. One of these antibodies is used as detection antibody which is conjugated with gold nanoparticles and deposited on conjugate pad. Whereas the other antibody is used as capture antibody and immobilized on test line. Anti-IgG antibody is used as control capture antibody.

This lateral flow immunoassay works as a sandwich assay in which, If the human red blood cells are present in the sample it will bind to the anti-erythrocyte antibodies conjugated to the gold nanoparticles and travel the length of the nitrocellulose strip and also bind to the another anti-erythrocyte antibody which is immobilized at the test line resulting in an antibody-gold nanoparticle-antigen-antibody complex of a red color. If the human red blood cells are not present, then there is nothing keeping the gold nanoparticles adhered to the test line and it will remain colorless. Antibody-

★ Star indicates an important underlined note for users to read while performing the assay

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conjugated gold nanoparticles will always bind at control line whether the antigen is present or not, which indicates that the assay is working correctly.

Stability and Storage

The kit can be stored at 2-8^{0}C. The test is stable up to the expiry date mentioned on the kit.

Sensitivity

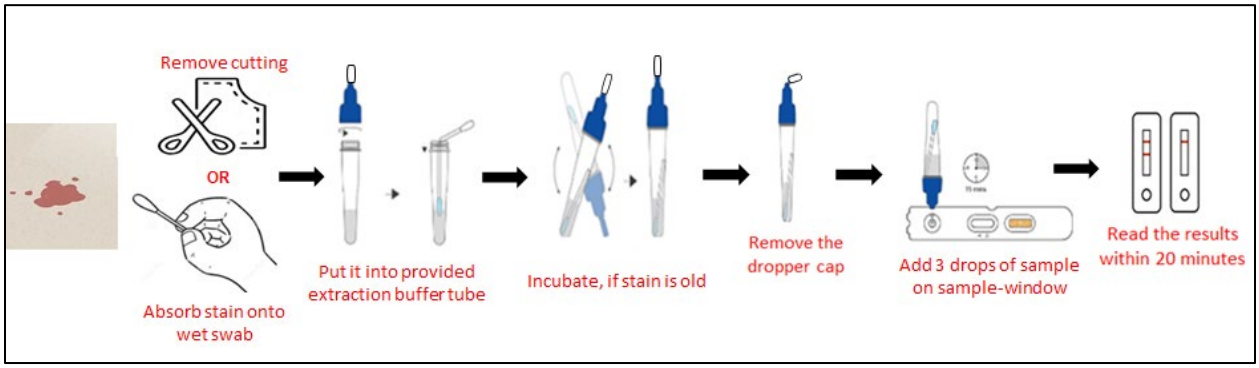
★ The kit can detect <u>less than 1.0 µl of human blood</u>. No high dose hook effect was seen in the developed kit.

Specificity

→ Insta-LF^{TM} Rapid-B kit specifically detects human blood. No cross reactivity has been observed with tested animal/bird blood i.e., Chicken, goat, pig, dog, cat, cow, buffalo, and horse.

→ No cross reactivity has been observed with tested human body fluids i.e., Saliva, urine, vaginal fluid, semen, and breast milk.

Schematic representation of a test run:



<u>Note</u>:

- Bring test device and sample at room temperature prior to assay
- The test cassette must remain in the sealed pouches until they are used
- Follow test instructions exactly. Even minor changes can affect test results
- Whole blood can not be added to the test device as a sample; due its viscosity, sample can not to pass through the membrane
- If control line appears, even a weak test line indicates positive test result

Protocol for Positive and Negative control

Positive control for Insta-LF^{TM} Rapid-B test can be produced as follows.

★ Put a drop of human blood (5-10µL) on a clean surface, to simulate crime scene <u>let it dry (at least for 10 minutes)</u>. Wet the sterile cotton swab with 2 drops of the Extra-extraction buffer provided in brown dropper bottle with the kit. Collect the blood sample from the surface on wet swab. Unscrew the purple cap of prefilled extraction buffer tube, cut the swab head, and put it into tube. Close the cap tightly, extract the blood by shaking the tube vigorously. Open the white dropper cap, add 3 drops of the extracted sample on sample window (round opening) of the cassette. Place the strip on flat surface and read the results exactly after 20 minutes.

Negative control for Insta-LF^{TM} Rapid-B test can be produced using sterile cotton swab (provided with the kit). Wet the sterile cotton swab with 2 drops of the Extra-extraction buffer provided in brown dropper bottle with the kit. Unscrew the purple cap of prefilled extraction buffer tube, cut the swab head, and put it into tube. Close the cap tightly, shake the tube vigorously. Open the white dropper cap, add 3 drops of the extracted sample on sample window (round opening) of the cassette. Place the strip on flat surface and read the results exactly after 20 minutes.

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Forensic Sample Preparation:

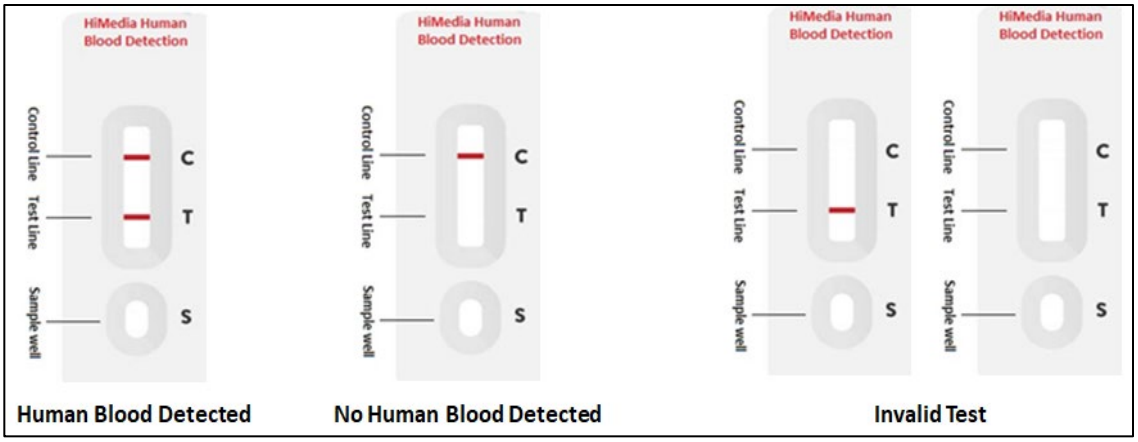
★ Wet Forensic Sample: Absorb 1-2 drops of sample on sterile cotton swab (provided with the kit). Unscrew the purple cap of prefilled extraction buffer tube, cut the swab head, and put it into tube. Close the cap tightly. Extract the blood by
★ shaking the tube vigorously. Wet sample does not require incubation in extraction buffer. Open the white dropper cap, add 3 drops of the extracted sample on sample window (round opening) of the cassette. Place the strip on flat surface and read the results exactly after 20 minutes.

★ Dried blood on the surface: Wet the sterile cotton swab with 2 drops of Extra-extraction buffer provided in brown dropper bottle with the kit. Collect the sample from the surface on the wet swab. Unscrew the purple cap of prefilled extraction buffer tube, cut the swab head, and put it into tube. Close the cap tightly. Extract the blood by shaking the tube vigorously.
★ (If the bloodstain is old, extract blood by incubating the extraction tube for 1 hour on shaker). Open the white dropper cap, add 3 drops of the extracted sample on sample window (round opening) of the cassette. Place the strip on flat surface and read the results exactly after 20 minutes.

★ Dried blood on cloth or paper: Take a cutting of stained part (≈ 4-6 mm). Unscrew the purple cap of the prefilled extraction
★ buffer tube, put the cutting into tube. Close the cap tightly. Incubate on shaker for 1 hour. Open the white dropper cap, add 3 drops of the extracted sample on sample window (round opening) of the cassette. Place the strip on flat surface and read the results exactly after 20 minutes.

Interpretation

- Two lines, one at Control Zone (C) and one at Test Zone (T), indicates that the test performed properly, and human blood detected. Even a weak test line indicates a positive test result
- Only one red line appears in the Control Zone (C), indicates that test performed properly but no human blood detected
- One red line always appears at Control Zone (C). If no red line appears in the Control Zone (C), the test is invalid - discard the test and repeat with new sample and new strip



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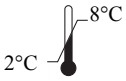

Storage temperature

Do not use if package is damaged

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