INTENDED USE

IH-Papain is a reagent for enzyme treatment of red blood cells for blood group antibody identification with the IH-Card AHG Anti-IgG.

SUMMARY

IH-Papain is intended for use in the two-stage enzyme technique where the red blood cells are pretreated with the enzyme and excess enzyme is removed by washing prior to the addition of serum or plasma. This is recognized as being more sensitive for the detection of antibodies than a one-stage technique whereby the enzyme is added directly to the red blood cell/serum mixture.

PRINCIPLES OF THE TEST

Papain is a proteolytic enzyme that modifies some human red blood cells by increasing the reactivity of some antigens particularly the antigens of the Rh, Kidd, and Lewis blood group systems. This enhanced reactivity is thought to be due to the reduction of sialic acid on the red cell membrane thus reducing the negative charge of the membrane. This reduces the natural repulsion between the antigens and antibodies, allowing for more antibody uptake by the red cells and in some cases, detection of IgG antibodies by direct agglutination. Conversely, proteolytic enzymes destroy or decrease the reactivity of some antigens like MNS1(M), MNS2(N), FY1(Fy^a), FY2 (Fy^b) and in some cases MNS3 (S) and MNS4 (s).

REAGENT

IH-Papain is extracted and prepared from Latex Carica Papaye. This extract is controlled in term of activity units and then lyophilized with suitable additives in order to ensure its performance and the absence of autolysis during storage.

IH-Papain is supplied in lyophilized form, and must be reconstituted with 5mL deionised water. The concentration of the enzyme in the reconstituted product is approximately 1%.

STORAGE REQUIREMENTS

• Store at 2 to 8 °C before reconstitution.
• Do not use beyond the expiry on the label, which is expressed as YYYY-MM-DD (Year-Month-Day).
• After opening, the reagent must be reconstituted immediately.
• After reconstitution, the reagents should be stored at 2 to 8 °C.
• After reconstitution, the reagent should be used within five (5) days of preparation.

PRECAUTIONS

• All IH-System reagents and test samples must be brought to room temperature (18 to 25 °C) prior to use.
• Do not use reagent if showing marked turbidity.
• Do not use damaged vials.

• Use reagent as furnished by following the instructions for use.
• Cards with dispersed drops observed at the top of the microtube, due to improper storage or shipping conditions, have to be centrifuged with the IH-Centrifuge L or IH-Reader 24 with preset time and speed before use. If drops are still observed on top of the microtube after one centrifugation it is recommended to not use the card.
• The use of volumes and/or red blood cell suspension in concentrations other than those indicated in the method, may modify the reaction and lead to incorrect test results, i.e., false positive or false negative results.
• Caution: The packaging of this product (cap) contains natural rubber latex which may cause allergic reactions.
• Warning: The IH-Card used with this reagent contains sodium azide, which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.
• Once the IH-Card has been used for testing, it may contain infectious material and should therefore be handled and disposed of as bio hazardous waste in accordance with local, state, and national regulations.

Hazard statements:
• H315 Causes skin irritation.
• H319 Causes serious eye irritation.
• H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
• H335 May cause respiratory irritation.

Precautionary statements:
• P261 Avoid breathing dust.
• P271 Use only outdoors or in a well-ventilated area.
• P285 In case of inadequate ventilation wear respiratory protection.
• P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
• P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
• P405 Store locked up.
• P501 Dispose of contents/container to hazardous or special waste collection point.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Blood samples should be collected following general blood sampling guidelines.

Samples should be centrifuged for 10 minutes at 2000g or at a time and speed that consistently produces a distinct cell/plasma interface. Donor segments do not require centrifugation.

Please refer to the appropriate IH-Card AHG Anti-IgG instructions for use for sample requirements.

TEST PROCEDURE FOR MANUAL AND AUTOMATED SYSTEMS

Material provided
• IH-Papain lyophilized, 5 mL vial

Materials required but not provided
• IH-Card AHG Anti-IgG
• Dispenser pipette capable of delivering 1 mL
• Pipettes: 10 µL, 100 µL, 900 µL, 1 mL and 5 mL
• Disposable pipette tips
• 37 +/- 1 ° C water bath
• Isotonic saline solution (0.85 to 0.90%)
• Sterile distilled water
• Glass or plastic test tubes
• IH-Centrifuge L or IH-Reader 24 to centrifuge the IH-Cards at 85g with pre-set time for manual working
• IH-Incubator L for manual working
• IH-1000 for fully automation after the manual treatment

Method for manual testing

A) Papain reconstitution
1. Add 5 mL of deionised water to the IH-Papain vial
2. Incubate 10 ± 1 min at 37 +/- 1 °C
3. Mix gently

B) Papain treatment of red blood cells
1. Wash the red blood cells three (3) times with isotonic saline solution (0.85 to 0.90%)
2. Prepare a red blood cell suspension of approximately 5% (e.g. 50 µL of packed red blood cells in 950 µL isotonic saline solution).
3. Add 1 volume of Papain to 9 volumes of red blood cell suspension (e.g. 100 µL of IH-Papain plus 900 µL red blood cell suspension).
4. Incubate for 5 minutes at 37 +/- 1 °C.
5. Wash three (3) times with isotonic saline solution.
   Note: A large amount of isotonic saline solution (at least 2 times the volume to wash) must be added immediately after incubation to stop / dilute enzymatic activity.
6. Prepare a red blood cell suspension of approximately 1% (e.g. 10 µL of packed washed papainized-red blood cells in 1 mL isotonic saline)
C) Test Procedures with specific IH-Cards.

1. For testing at 37 +/- 1 °C, use the 1% suspension in isotonic saline and refer to the Antibody Identification test procedure as described in the IH-AHG Anti-IgG Card instructions for use.
   *Note: Suspension of papain treated cells in IH-LISS Solutionlead to increased unexpected positive results and has not to be used.*

2. For specific instructions for the Indirect Antiglobulin Test, please refer to the test procedure as described in the IH-AHG Anti-IgG Card instructions for use.

**INTERPRETATION OF RESULTS**

Please refer to the appropriate instructions for use for the specific IH-Card used

**STABILITY OF REACTIONS**

Please refer to the appropriate instructions for use for the specific IH-Card used

**QUALITY CONTROL**

On each day of use, the reactivity of all Blood Grouping Reagents should be confirmed by testing with known positive and negative samples. Each reagent is satisfactory for use if positive and negative samples react as expected.

**LIMITATIONS**

Erroneous and abnormal results may be caused by:
- Bacterial or chemical contamination of the blood specimens, reagents, supplementary materials and/or equipment.
- Patient medication or disease yielding a cross-reaction.
- A red blood cell concentration or suspension medium different from that recommended.
- Incomplete resuspension of the red blood cells.
- Sample hemolysis prior to testing.

- Use of procedure other than the one described above.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Fibrin, clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the gel and may cause an anomalous result. They may appear as a pinkish layer. In a negative reaction the false appearance of a mixed field could lead to misinterpretation. It is recommended to re-clot the serum and repeat the test.
- If red blood cells (pellet at the bottom of the microtube) are too low in concentration, they become difficult to visualize, and, in certain cases a weak positive reaction can fail to be detected.

Please refer to the IH-Centrifuge L and IH-Incubator L User Manual NA for instrument specific assay limitations.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

The final release testing is performed according to the product-specific Standard Operating Procedures. As part of the lot release process, each lot of Bio-Rad Blood Grouping Reagents is tested against antigen positive and negative samples to ensure suitable reactivity and specificity.

For technical support or further product information, contact Bio-Rad Laboratories, Inc. at 800-224-6723.

**GLOSSARY OF SYMBOLS**

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<th>Symbol</th>
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<tr>
<td>LOT</td>
<td>Batch Code</td>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
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**BIBLIOGRAPHY**
