CE

LIQUIPAP

STABILIZED, ACTIVATED PAPAIN ENZYME SOLUTION FOR SEROLOGICAL APPLICATIONS

SUMMARY

Enzyme treatment enhances the reactivity of red blood cells with certain antibodies of Rh, Kidd, Lewis and P systems. Certain clinically significant antibodies of Rh and Kidd systems can be detected only with enzyme treated cells.
Traditionally papain needs to be prepared fresh for use and long term storage at -20°C is recommended. This leads to frequent reagent preparation, lot to lot variation and strict quality control to assess adequate and correct performance. Stabilized papain solution overcomes this limitation. Thus an activated stabilized papain enzyme solution is useful in detecting clinically significant antibodies for specific serological studies. Proteolytic activity of papain destroys blood group antigens notably M, N, S, Fy^a, and Fy^b, a property which may be useful for the identification and separation of mixed antibodies.

PRESENTATION

REF	10250005
Pack	5 ml

REAGENT

LIQUIPAP is a stabilized ready to use papain reagent useful for serological applications such as antibody screening, antibody detection and cross match techniques.

REAGENT STORAGE AND STABILITY

Store the reagent at 2-8°C. DO NOT FREEZE.
The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

The sialic acid molecules present in the red cell membrane impart a net negative charge to the surface of the red cell. Due to the negative charge a repulsive force exists between two red blood cells, which is termed as the 'zeta potential'.
Proteolytic enzymes such as papain reduce the red blood cell surface charge by cleaving the sialic acid molecules from the polysaccharide chains on the red blood cell membrane. Also the enzyme treatment causes spicule formation on the red cell thereby exposing the red blood cell antigens on the surface. This dual action of reduction in the 'zeta potential' and exposure of the red blood cell antigens on the surface enhances the agglutination reaction.

NOTE

1. *In vitro* diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains 0.1% sodium azide as a preservative. Avoid contamination with skin and mucosa. On disposal flush with large quantities of water.
3. Do not freeze or expose the reagent to elevated temperatures. After usage immediately replace the reagent vial back to 2-8°C.
4. Marked turbidity may indicate reagent deterioration or contamination, such reagent should not be used.
5. Do not use the damaged or leaking reagents.

SAMPLE COLLECTION AND PREPARATION

No special preparation is required prior to sample collection by approved techniques. Serum samples may be stored at 2-8°C upto 3 hours if not tested immediately. Do not use haemolysed samples.
Red blood cells used for detecting antibodies, should preferably be fresh.

ADDITIONAL MATERIAL REQUIRED

Test tubes (12 x 75 mm), Pasteur pipettes, laboratory centrifuge, incubator (37°C), isotonic saline/ isotonic buffered saline, donor red blood cells and recipient serum for cross match, reagent red blood cells for antibody detection, optical aid.

PROCEDURE

Bring all the reagents to room temperature (25-30°C) before testing.

One stage test

A) For cross match

1. Wash the donor red blood cells to be tested atleast three times in isotonic saline.
2. Prepare 2-3% red blood cell (donor) suspension in isotonic saline.
3. To an appropriately labelled test tube add two drops of recipient serum to be tested and two drops of donor red blood cell suspension. Mix the contents thoroughly but gently.

4. Immediately add two drops of LIQUIPAP reagent.
5. Incubate at 37°C for 15-30 minutes.
6. Centrifuge at 1000 rpm for 2 minutes.
7. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

B) For antibody detection

1. Wash the reagent red blood cells to be tested atleast three times in isotonic saline.
2. Prepare 2-3% reagent red blood cell suspension to be tested in isotonic saline.
3. To an appropriately labelled test tube add two drops of serum to be tested and two drops of reagent red blood cell suspension under test.
4. Mix the contents and immediately add two drops of LIQUIPAP reagent.
5. Incubate at 37°C for 15-30 minutes.
6. Centrifuge at 1000 rpm for 2 minutes.
7. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

Alternatively a two-stage test using LIQUIPAP reagent can also be performed as follows.

Two stage test

A) For Cross match

1. Wash the donor red blood cells three times in isotonic saline.
2. To an appropriately labelled test tube add one drop of washed packed cells (donor) and one drop of LIQUIPAP reagent.
3. Incubate the test tube at 37°C for 15-30 minutes.
4. Wash the LIQUIPAP treated donor red blood cells atleast three times with isotonic saline.
5. Prepare LIQUIPAP treated 2-3% red blood cell suspension of donor in isotonic saline.
6. To an appropriately labelled test tube add one drop of LIQUIPAP treated 2-3% donor red blood cell suspension.
7. Add two drops of recipient serum to be tested.
8. Mix well and incubate at 37°C for 30 minutes.
9. Centrifuge at 1000 rpm for 2 minutes.
10. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

B) For antibody detection

1. Wash the reagent red blood cells to be tested three times in isotonic saline.
2. To an appropriately labelled test tube add one drop of washed packed reagent red blood cells under test and one drop of LIQUIPAP reagent.
3. Incubate the test tube at 37°C for 15-30 minutes.
4. Wash the LIQUIPAP treated reagent red blood cells under test atleast three times with isotonic saline.
5. Prepare LIQUIPAP treated 2-3% reagent red blood cell suspension under test in isotonic saline.
6. To an appropriately labelled test tube add one drop of LIQUIPAP treated 2-3% reagent red blood cell suspension under test.
7. Add two drops of serum to be tested.
8. Mix well and incubate at 37°C for 30 minutes.
9. Centrifuge at 1000 rpm for 2 minutes.
10. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

INTERPRETATION OF RESULTS

Agglutination and / or haemolysis indicates an antibody directed against the antigen present on the red blood cell under test.
No agglutination and/or haemolysis indicates absence of enzyme reactive antibodies directed against the antigen present on the red blood cell under test.

REMARKS

1. As under centrifugation or over centrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required for achieving the desired results.
2. Erroneous results may also occur due to improper red blood cell concentration, improper incubation time or temperature while performing the test.
3. All enzyme preparations are subject to some loss of potency, it is therefore recommended to check the reagent performance with known negative control (neutral AB serum) and positive control (Coombs control cells) on a regular basis.
4. The ability of papain to denature IgG molecule renders the one stage technique less sensitive though it is a convenient method for use in cross match techniques.
5. Papain is not suited for the detection of Anti-M, -N, -S, -Duffy since the corresponding antigens are destroyed during the proteolytic action of papain enzyme.
6. LIQUIPAP reagent is a colourless to pale yellow clear solution. Repeated exposure to elevated temperatures may impart a dark colour to the reagent. In such cases the reagent performance must be assessed closely before use.

7. Usage of isotonic buffered saline while performing the test ensures in maintaining the optimum pH of the reaction milieu for antigen antibody reaction.
8. Alternatively LISS (Low ionic strength solution) can be used while performing the test. LISS lowers the ionic concentration of the reaction milieu thereby potentiating the rate of antibody uptake by the antigen present on the red blood cell membrane.
9. It is recommended to run a control with each assay series.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty for use and sale for any other purpose.

BIBLIOGRAPHY

1. Blood Transfusion in Clinical Medicine, PL Mollison, CP Engelfriet, Marcela Contreras, 9th Edition, 1994, Blackwell Science Publications.
2. AABB Technical Manual, 13th Edition, 1999.
3. Clinical Diagnosis and Management by Laboratory methods, John Bernard Henry, 17th Edition, 1984, W B Saunders Company.
4. Data on File: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

 Temperature limitation	 Manufacturer	LOT Batch Number/ Lot Number	 This side up
 Use by	 Consult Instructions for use	IVD *In vitro* Diagnostic Medical Device	 Contains sufficient for <n> tests
 Date of Manufacture	REF Catalogue Number	EC REP Authorised Representative in the European Community	

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