TULIP DIAGNOSTICS (P) LTD.

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LYOPAP

REF	102500061
Pack Size	6 x 1 ml

LYOPHILIZED PAPAIN FOR SEROLOGICAL APPLICATIONS

SUMMARY
Along with Coombs techniques, Saline and Enzyme techniques are also very important to detect antibodies which predominantly react at 4°C or at room temperature. Enzyme techniques are very useful when increased sensitivity in detecting an antibody is required. Enzymes enhances the reactions of certain antibodies like Rh, Kell and Kidd system and at the same time some antigens like M, N, S of MNS system and Fya and Fyb of Duffy system are destroyed by Enzyme treatment.
LYOPAP can be used for conventional serological applications and for applications on Matrix™ Gel System.

REAGENT
LYOPAP is a lyophilized papain for serological applications.

REAGENT STORAGE AND STABILITY
Store the reagent at 2-8°C. DO NOT FREEZE.
The shelf life of the unopened reagent vial is as per the expiry date mentioned on the reagent vial. Vial once opened or reconstituted with distilled water is stable for 7 days at 2-8°C.

PRINCIPLE
The sialic acid molecules present on 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 contact 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. Do not use the reagent beyond expiry date.

SAMPLE COLLECTION AND PREPARATION
No special preparation of patient is required prior to sample collection by approved techniques. For optimum results use freshly collected sample. Anticoagulants like EDTA, CPD-A and Citrate can be used. Do not use haemolysed samples.

PROCEDURE
Bring all the reagents to room temperature before testing.

PREPARATION OF THE REAGENT
1. Reconstitute the LYOPAP vial with 1 ml of sterile bi-distilled water. Avoid using water containing preservatives.
2. Re-stopper the vial and allow to stand until the hydration is complete (usually 5-7 minutes).
3. Mix by gently swirling and inversion, avoid froth formation. Do not shake.
4. Allow to stand and equilibrate for a further 20 minutes before use.
5. Reconstituted LYOPAP is now ready for use.

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 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.

4. Mix the contents thoroughly but gently.
5. Immediately add two drops of LYOPAP reagent.
6. Incubate at 37°C for 15-30 minutes.
7. Centrifuge at 1000 rpm for 1 minute.
8. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

B) For Antibody Screening / Identification

1. To an appropriately labelled test tube add two drops of serum to be tested and two drops of 3% reagent red blood cell suspension under test.
2. Mix the contents and immediately add two drops of LYOPAP reagent.
3. Incubate at 37°C for 15-30 minutes.
4. Centrifuge at 1000 rpm for 1 minute.
5. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

Alternatively a two-stage test using LYOPAP 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 LYOPAP reagent.
3. Incubate the test tube at 37°C for 15-30 minutes.
4. Wash the LYOPAP treated donor red blood cells atleast three times with isotonic saline.
5. Prepare 3% red cell suspension of LYOPAP treated donor red cells in isotonic saline.
6. To an appropriately labelled test tube add one drop of LYOPAP treated 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 1 minute.
10. Gently resuspend and observe for agglutination and/or haemolysis macroscopically and microscopically.

B) For Antibody Screening / Identification

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

Note: For applications of Matrix™ Gel System use as per the instructions in respective card package insert.

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 no 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. 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.
4. 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.
5. Usage of isotonic buffered saline while performing the test ensures in maintaining the optimum pH of the reaction milieu for antigen antibody reaction. Alternatively LISS (Low ionic strength solution) can be used while performing the test. LISS lowers the ionic concentration reaction milieu thereby potentiating the rate of antibody uptake by the antigen present on the red blood cell membrane.
6. 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

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2. AABB Technical Manual, 15th Edition, 2005.
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.