

**BOVINE SERUM ALBUMIN**  
22% SOLUTION FOR SEROLOGICAL APPLICATIONS

**SUMMARY**

Bovine Serum Albumin is mainly used to enhance the reactivity of blood grouping and typing antibodies in direct agglutination tests. Bovine Albumin also enhances the reactivity and sensitivity of indirect antiglobulin test which is used for compatibility testing, antibody screening, identification and titration.

**REAGENT**

Bovine Serum Albumin is manufactured from selected raw Bovine serum, its protein concentration and pH adjusted to 22% and 7.1(± 0.1) respectively. Its conductivity is controlled specifically for serological applications.

**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**

Agglutination of antibody coated red cells depends upon the class and type of antibody involved and the characteristics of the reaction medium such as ionic strength and pH.

Incomplete antibodies of IgG class, especially those with Rh specificity, agglutinate red cells if the zeta potential between the red cells is adjusted by addition of colloids and salts such as Bovine Serum Albumin reagent. Addition of BSA enhances such immunological reactions and increases test sensitivity.

**NOTE**

(1) In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use. (2) The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. (3) Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.

**SAMPLE COLLECTION AND PREPARATION**

No special preparation of the patient is required prior to sample collection by approved techniques.

Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed samples.

Donor units can be tested upto the end of their dating.

For the indirect antiglobulin test, serum from fresh clotted whole blood should be used.

**ADDITIONAL MATERIAL REQUIRED FOR COMPATIBILITY TESTING**

Test tubes (10 x 75 mm), 0.2 ml serological pipettes, Pasteur pipettes, Human red blood cells with specific antigen reacting with the antibody to be titrated, Centrifuge, Incubator, Isotonic saline, Anti human globulin reagent such as Anti human Globulin Reagent, Coombs control cells: (Refer Anti human Globulin pack insert), AB Neutral human serum.

**BROAD SPECTRUM COMPATIBILITY TEST**

**Major cross match procedure**

Initial Phase

1. Label two test tubes as A (for albumin) and B (for saline); depending upon the number of donors to be cross matched, as many pairs of such labelled tubes would be required.
2. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
3. Pipette two drops of recipient serum in both the labelled test tubes.
4. Pipette one drop of donor red cells in both the labelled test tubes and mix well.
5. Only to the albumin tube (A) add two drops of Bovine Serum Albumin reagent and mix well.
6. Centrifuge both the tubes for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 rpm (1000 g).
7. First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically.
8. Proceed to incubation phase.

### Incubation Phase

1. Incubate the saline tube at room temperature and the albumin tube at 37°C for fifteen minutes.
2. First observe for haemolysis. Resuspend the cell button and observe for agglutination macroscopically.
3. Proceed to the antiglobulin phase.

### Antiglobulin Phase

1. Only the albumin tubes (A) are tested in the antiglobulin phase.
2. Wash the mixture of red blood cells and serum thoroughly with isotonic saline for a minimum of three times. Decant completely after the last wash.
3. Place two drops of Anti Human Globulin reagent into the test tube containing the sedimented cells and mix well.
4. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Very gently, resuspend the cells and observe for agglutination macroscopically.

### **Antibody Titration Test**

1. a) Prepare a 5% suspension of red blood cells with specific antigen reacting with antibody to be titrated, in Bovine Serum Albumin reagent.  
b) Also prepare a 5% suspension of patients red cells in Bovine Serum Albumin reagent.
2. Label ten test tubes (1 to 10) and make progressive dilutions of the patient serum as indicated below:
  - a) Pipette 0.1 ml of AB Neutral serum into each test tube except the first tube.
  - b) Pipette 0.1 ml of the patient serum into first two tubes only.
  - c) After mixing the contents of the second tube thoroughly, transfer 0.1 ml of the mixture to the third tube. Continue the serial dilution by transfer upto tube No. Ten, Discard 0.1 ml of the mixture from the last tube.
3. To tubes No. One thru' to Nine, add one drop of Albumin suspended selected red blood cells, (as prepared in point No. 1 (a) above) and mix well.
4. To tube No. Ten add one drop of patient red cells suspended in albumin (as prepared in point No.1 (b) above) and mix well.
5. Incubate all the tubes at 37°C for a minimum of fifteen minutes.
6. Centrifuge all the tubes for one minute at 1000 rpm or 20 seconds at 3400 rpm (1000 g).
7. Very gently, resuspend the cell buttons and observe for agglutination macroscopically.
8. Antiglobulin test should be performed on all tubes which do not show a very strong agglutination.

### **INTERPRETATION OF RESULTS**

#### **Compatibility Test**

In all phases of the compatibility test, if no agglutination or haemolysis is observed then the patient and the donor may be considered compatible.

If haemolysis or agglutination at any point till the completion of the antiglobulin phase is observed the patient and donor are considered incompatible.

#### **Antibody Titration Test**

The end point of the titration is the reciprocal of the dilution in the last tube showing agglutination.

### **REMARKS**

(1) If plasma is used in the indirect antiglobulin test, the complement dependent antibodies may not be detected due to the absence of calcium. (2) To all negative test results, after the antiglobulin test phase, one drop of Coombs control cells should be added. If the Coombs control cells do not agglutinate, then the compatibility test must be repeated. (3) Red blood cells showing a positive direct antiglobulin test should not be used for the indirect antiglobulin test. (4) Bovine serum albumin will not bring about agglutination of red cells by all IgG blood grouping typing antibodies. (5) As undercentrifugation or overcentrifugation can lead to erroneous results, it is recommended that each laboratory calibrate its own equipments and the time required for achieving the desired results. (5) After usage the reagent should be immediately recapped and replaced to 2-8°C storage.

### **WARRANTY**

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

**BIBLIOGRAPHY**

(1) Lee H.H. Rouger P., Germain C., Muller A. & Salmon C. (1983). The production and standardisation of monoclonal antibodies as AB blood group typing reagents Symposium of International Association of Biological Standardisation on monoclonal antibodies. (2 Human Blood Groups by Geoff Daniels, 1<sup>st</sup> Ed., Blackwell Science, Oxford, 1995. (3) HMSO, Guidelines for the Blood Transfusion Services, 2<sup>nd</sup> Ed., 1994.

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