BOVINE ALBUMIN 30%

CONTENTS			
REF	3406010	Bovine Albumin 30%	10 mL
For *in vitro* diagnostic use only			

BSA
Bovine Albumin 30%

Qualitative procedure for use in antibody detection, identification and titration and as an antiserum diluent.
SLIDE AND TUBE TESTS

PRINCIPLE

Linear Chemicals Bovine Albumin 30% is useful adjunts utilized in various immunohematologic applications as a potentiater in the reaction between antibodies of small size (IgG class) and their antigens.
Albumin acts by decreasing the zeta potential and raising the dielectric constant of the medium, thus reducing the repulsive force between cells and favouring agglutination.
Rhesus antibodies react well in a reaction environment containing Bovine serum albumin (BSA).

APPLICATIONS

- Screening for unexpected antibodies
- Compatibility testing
- Identification and titration of antibodies (The procedure determines the potency of antibodies resulting from pregnancy and transfusion reactions).
- Antigen identification procedure

REAGENTS COMPOSITION

Albumin bovine 30% has been prepared by fractionating bovine serum.

Bovine albumin 30%	Bovine Albumin solution to 30% in PBS. pH 6.5-7.4 Sodium azide 0.95 g/L.

Precautions: Although the bovine serum has been tested for infectious diseases and found negative, the reagent cannot be assumed to be free from infectious agents. However, should be handled cautiously as potentially infectious.
Protective clothing should be worn when handling the reagent, such as disposable gloves.

Warning: The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

REAGENT PREPARATION

The reagents are ready to use.

STORAGE AND STABILITY

1. The reagents will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use the reagents after the expiration date.
2. Do not freeze or expose to elevated temperatures. Prolonged storage outside the recommended temperature range may result in accelerated loss of reagent reactivity.
3. This product should be clear. Turbidity may indicate microbial contamination. Do not use the reagents if a precipitate is present.
4. If a vial is cracked or leaking, discard the contents immediately.

SAMPLES

- Fresh Red cells (Antigen identification). Free of haemolysis. Store at 2º-8ºC.
- Serums less than 24 hours old kept at 2-8ºC since separation from the clot (Compatibility testing, screening, identification and titration of antibodies)

MATERIAL REQUIRED

Slide test
- Glass slide.
- Applicaror stick.
- Rh viewbox (45-50ºC).

Tube test
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Pasteur pipettes.
- Water bath at 37ºC.
- Centrifuge Sero-fuge or similar.

ADDITIONAL REAGENT REQUIRED

- Phosphate Buffered Saline (PBS):
  8.5 to 9.0 g/L NaCl (0.145-0.154 mol/L) pH 7.0±0.2 at 22 ±1ºC.
- Test red cells. (See the instrucctions of the manufacturer).

PROCEDURE

I. Crossmatch. Slide Test

1. Resuspend the sample of whole blood from the donor (appox. 35-40% cell concentration)
2. Place on a slide:
   - 1 drop whole blood
   - 1 drop of recipient's serum.
   - 2 droops of Bovine Albumin 30%.
3. Mix well and spread the cell-serum mixture over the slide.
4. Place the slide on a prewarmed (45º-50ºC) lighted viewing box.
5. Tilt viewing box slowly back and forth for 2 minutes observing macroscopically for agglutination.
6. **Reading**
   Examine macroscopically for agglutination.

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II. Indirect Antiglobulin Test. Tube test

1. Prepare a 3-5% of red blood cells suspension in PBS. Comercial cells should be use as supplier.
2. Place in a glass test tube:
   - 1 drop of cell suspension
   - 2 drops of patient serum
   - 3 drops of Bovine Serum
3. Mix well and centrifuge 20 seconds at 1000 r.c.f. or for a suitable alternative time and force.
4. Resuspend the cells by gently tilting or shaking the tube and examine macroscopically for agglutination (Note 1,2).
5. Mix and incubate at 37ºC for 30 min.
6. Centrifuge, see step 3 and examine the presence or absence of agglutination, see step 4.
7. Wash the cells three times with PBS.
   Wash (Note 3): fill the tube with PBS (approx. 4mL) and centrifuge the red cells allow adequate spin time sediment red cells, decant completely the supernatant. Repeat the wash twice. Decant completely after the last washing.
8. Add 1 drop of ANTI-HUMAN GLOBULIN, mix well.
9. Centrifuge (see step 3) and examine the presence or absence of agglutination (step 4; Note 4).

Interpretation

A positive result, presence of agglutination, indicates that red cell antibodies against one or more specific antigens on the red blood cells concerned are present

QUALITY CONTROLS AND ADVICE

Use of an autocontrol is recommended with each procedure using recipient's cells and his serum.

NOTES

Note 1. Read all tube tests after centrifugation because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.

Note 2. Haemolysis indicates the presence of complement-binding antibodies.

Note 3. Complete washing steps without interruption.

Note 4. To confirm the validity of a negative result one-drop (50 µL) of washed cells coated with a weak IgG antibody (Coombs Control Cells) should be added to the tube, recentrifuged and examined for agglutination (See: Procedure. Tube Test. Step 3 and 4) If no agglutination is observed the test is invalid and must be repeated.

ANALYTICAL PERFORMANCE

- The reagent has been characterized by all the procedures mentioned in the PROCEDURE and shows to enhance agglutination with red blood coating with anti-IgG.
- Each lot is tested to assure specificity in antibody-free system with red cells know to possess the most frequently inherited blood group antigens.

LIMITATIONS OF THE PROCEDURE

- The Bovine Albumin 30% not enhance the reactivity of all blood group antibodies.
- Inadequate washing of the red cells, contamination with human serum protein of the anti-serum, incorrect incubation or centrifugation times and inadequate temperature ranges give false negative reactions.
- Red cells sensitized with an autoantibodiy *in vitro* or *in vivo* may agglutinate spontaneously in concentrations of Bovine albumin as low as 6%.
- Test serum should be stored no longer than 24 hours at 2-8ºC or 1 month at –20ºC in order to detect complement-binding antibodies.
- No single test is capable of detecting all clinically significant antibodies.
- The user must determine the suitability of the reagents for use in other techniques.
- Use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use.

REFERENCES

1. Diamond, l.k. and Denton, R.L. : J. Lab. Clin Med., 30 , 821 (1945)
2. Pollack, W.: Transfusion (Philadelphia), 4, 411 (1964)

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