














SBio Anti-Human Globulin

For Direct and Indirect Antiglobulin Tests

REF	90180610	90180010
Pack	6 x 10 ml	10 ml

 Store at 2-8°C	 Manufacturer	 Batch Number	 Description of reagent
 Use by (Last day of stated month)	 Consult instructions for use	 This side up	 Authorised Representative
 Date of Manufacture	 Catalogue Number	 In vitro Diagnostic Medical Device	 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions. 



SUMMARY

Generally antibodies involved in transfusion reactions are of two types, namely the complete and the incomplete, whereas the complete antibodies agglutinate red cells in saline medium, the incomplete type of antibody sensitizes red cells without agglutination. Usually IgM class of antibodies and IgG, and IgG, type of IgG antibodies fix complement. Cell lysis, *in vivo*, is mediated through the complement system and the complement component C_{3b} is further acted upon to produce C_{3d}.

In the direct antiglobulin tests, Anti-Human Globulin Reagent is used to detect antibodies adsorbed to the red blood cells *in vivo*.

In the indirect antiglobulin tests, Anti-Human Globulin Reagent is used to detect antibodies adsorbed to the red blood cells *in vitro*.

Anti-Human Globulin Reagent is useful for compatibility testing, antibody detection, antibody identification, umbilical cord red blood testing and detection of the D⁺ variant of the human red blood cell antigen D (Rho).

REAGENT

SBio Anti-Human Globulin is a balanced ready to use blend of highly purified immunoglobulins. It contains Anti-Human IgG antibodies and antibodies reactive with human complement components C_{3d}. These anti complement antibodies are IgM class monoclonals and they impart the necessary sensitivity to the reagent.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and titre.

REAGENT STORAGE AND STABILITY

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

PRINCIPLE

Normal human red blood cells, in presence of antibody directed towards the antigen they possess, may fail to agglutinate and become sensitized. This may be due to the particular nature of the antigen and antibody involved. SBio Anti-Human Globulin Reagent would react with red cells sensitized with gamma globulins or components of human complement involved and cause agglutination of the red blood cells.

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. (4) SBio reagents are not from human source, hence contamination due to HBsAg and HIV is practically excluded. (5) It is necessary to use the calibrated dropper provided in the reagent vial to dispense a reagent drop. (6) It is advisable to wear gloves and safety spectacles and handle test specimens of human origin with caution. (7) Do not use damaged or leaking reagents.

(8) Special protective measures, conditions for disposal and disinfection should be implemented in accordance with local regulations.

SAMPLE COLLECTION AND STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed samples.

For Direct Antiglobulin Test: Blood drawn into EDTA is preferred but oxalated, citrated or clotted whole blood may be used. The blood sample should be tested as soon as possible after collection and should not be stored.

For Indirect Antiglobulin Test: Serum, not more than 48 hours old, should be used. Donor units may be tested upto the end of their dating.

PREPARATION OF COOMBS CONTROL CELLS

(1) Dilute Anti-D (IgG) reagent 1:50 in isotonic saline. (2) Prepare a 5% suspension of group 'O' RhoD positive cells in isotonic saline. (3) Mix equal volumes of diluted Anti-D reagent (as in 1 above) and 5% suspension of 'O' RhoD positive cells (as in 2 above) and incubate at 37°C for 15 minutes. (4) Decant and wash thoroughly with isotonic saline atleast thrice. (5) Resuspend in isotonic saline to make a 5% suspension of coombs control cells.

ADDITIONAL MATERIAL REQUIRED

For Direct Antiglobulin Test: Test tubes (12 x 75 mm), Pasteur pipettes, Centrifuge, Isotonic saline, Coombs control cells, Optical aid.

For Indirect Antiglobulin Test and Compatibility Test: Test tubes (12 x 75 mm), Pasteur pipettes, SBio Bovine Serum Albumin, Centrifuge, Incubator (37°C), Isotonic saline, Coombs control cells, Optical aid.

PROCEDURE

Bring reagent to room temperature before testing.

Direct Antiglobulin Test

(1) Prepare a 5% suspension of the red cells to be tested in isotonic saline. (2) Pipette one drop of the cell suspension into a test tube. (3) Fill the tube with fresh isotonic saline and centrifuge for 30 seconds at 3400 RPM (1000g). (4) Decant and repeat this washing atleast thrice. (5) Add two drops of SBio Anti-Human Globulin Reagent and mix well. (6) Centrifuge for one minute at 1000 rpm (125 g) or for 20 seconds at 3400 RPM (1000 g). (7) Very gently, resuspend the cell button observing for agglutination macroscopically. (8) To all negative antiglobulin tests add one drop of coombs control cells and observe for agglutination.

Indirect Antiglobulin 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 SBio Bovine Serum Albumin reagent and mix well. (6) Centrifuge both the tubes for one minute at 1000 RPM (125g) or for 20 seconds at 3400 RPM (1000 g). (7) First observe for haemolysis. Resuspend the cell button, 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, 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 minimum of three times. Decant completely after the last wash. (3) Place two drops of SBio Anti-Human Globulin Reagent into the test tubes containing the sedimented cells and mix well. (4) Centrifuge for one minute at 1000 RPM (125 g) or for 20 seconds at 3400 RPM (1000 g). (5) Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Direct Antiglobulin Phase

Agglutination of red blood cells is a positive test result and indicates presence of human IgG or components of complement on the red blood cells.

No agglutination is a negative test result and indicates absence of human IgG or components of complement on the red blood cells.

Indirect Antiglobulin Phase

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 the donor are considered incompatible.

PERFORMANCE CHARACTERISTICS

Over 500 tests were conducted on a panel of random samples (Blood donors, patients and neonates) drawn on the recommended anticoagulants using the IVDMD intended for erythrocytic Resus D (RH1) phenotyping in the indirect antiglobulin test combined with SBio Anti-Human Globulin, Anti-D IgG and SBio Anti-D (IgM+IgG) showed 100% reactivity and specificity vis-a-vis common Resus D (RH1) phenotypes.

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 Coombs control cells do not agglutinate then the compatibility test must be repeated. (3) In the indirect antiglobulin test procedure an auto control tube (individual's cells in his own serum) should be run. (4) Red blood cells showing a positive direct antiglobulin test cannot be used for the indirect antiglobulin test. (5) It is recommended that Anti-IgG activity of the Anti-Human Globulin Reagent be tested from time to time preferably on a daily basis using Coombs control cells as a positive control. (6) All glassware used in the test should be scrupulously clean dry and free from contamination with human serum. (7) Contaminated Bovine Serum Albumin, saline or glassware may inactivate Anti-Human Globulin Reagent. (8) Use of various drugs and certain diseases (such as megaloblastic anaemia) are known to be associated with a positive direct antiglobulin test. (9) Cord cells obtained from a newborn exhibiting haemolytic disease of the newborn, especially due to ABO incompatibility may give false negative results. (10) SBio Anti-Human Globulin Reagent does not contain Anti-C₄ and is free from Anti-T activity. (11) As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required for achieving the desired results.

WARRANTY

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

BIBLIOGRAPHY

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

Manufactured by:

TULIP DIAGNOSTICS (P) LTD.

Plot Nos. 92/96, Phase II C, Verna Industrial Estate,
Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh,
Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.
Website: www.tulipgroup.com Email: sales@tulipgroup.com

Manufactured for:

Singapore 
Biosciences PTE Ltd.

11 Yishun Street 51, #04-23, The Criterion,
Singapore 767971

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EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain