

BIOSCOT®

Anti-Human Globulin Polyspecific

Anti-IgG/C3d



IVD

REF TS-10X10ML-B

Rabbit Serum/Cell Line BRIC-8

For Use in Antiglobulin Tube Techniques

INTENDED USE

BIOSCOT® Anti-Human Globulin polyspecific IgG/C3d blood grouping reagent (cell line BRIC-8) is used to ensure the immunological compatibility of blood and blood components intended for transfusion. This qualitative reagent will detect the presence of sensitising (but not directly agglutinating) blood grouping antibodies. The reagent is intended for use with non-automated manual direct and indirect antiglobulin tube techniques. The reagent is designed for in vitro diagnostic, professional use by operators trained in serological techniques.

APPLICATIONS

Indirect Antiglobulin Technique

- In screening the serum of blood donors and patients for antibodies
- In compatibility testing prior to blood transfusion
- In red cell phenotyping
- In the identification and titration of antibodies found in sera or eluates.

Direct Antiglobulin Technique

- In the laboratory diagnosis of haemolytic anaemia
- In the laboratory diagnosis of haemolytic disease of the newborn
- In the investigation of suspected transfusion reactions
- In the investigation of those autoimmune disorders involving binding of immunoglobulin and/or complement to red blood cells.

Note:

The detection of some clinically significant antibodies which activate complement (usually within the Kidd system) is enhanced and occasionally only possible through the use of a polyspecific Anti-Human Globulin reagent rather than monospecific Anti-IgG. The importance of the red cell diluent/washing solution is often underestimated. Phosphate buffered saline (PBS) pH 6.8-7.2 is preferable to unbuffered normal ionic strength saline.

PRINCIPLE OF THE REAGENT

The addition of Anti-Human Globulin to thoroughly washed red cells which are coated with antibody (immunoglobulin) and/or fragments of the third component of the complement system (C3b, C3bi, C3dg or C3d) will generally result in clearly visible agglutination of the red blood cells. BIOSCOT® Anti-Human Globulin (polyspecific) is a blend of selected dilutions of sera obtained from rabbits immunised with purified human IgG and murine monoclonal IgM Anti-C3d (cell line BRIC-8). The reagent has been standardised to give optimal detection of human IgG (all four sub-classes) and C3 fragments bound to red cells in all the routine diagnostic applications where direct or indirect antiglobulin techniques are appropriate. The reagent will not agglutinate red cells coated with C4d fragments.

The reagent has been characterised by the procedures recommended in these instructions for use, its suitability for use in other techniques must be determined by the user.

PRECAUTIONS

- The cell line used to produce this reagent is of murine origin and has been tested and found to be negative for Mouse Antibody Production (MAP) viruses. The human donors used in the manufacture of the rabbit antiserum have been tested and found to be negative for Anti-HIV1, Anti-HIV2, Anti-HCV, HBsAg and syphilis. Care must be taken in the use and disposal of each container and its contents.
- The reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead or copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

- This product should be clear. Turbidity may indicate bacterial contamination. The reagent should not be used if a precipitate, fibrin gel or particles are present.
- The bovine material is obtained from USDA approved sources or from sources for which origin information is available. The donor animals have been inspected and certified disease free and are deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.
- The product should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

CONTROLS

To confirm the validity of a negative result one drop of IgG sensitised red cells (Coombs Control cells) should be added to the tube, recentrifuged and examined for agglutination. If no agglutination is observed the test is invalid and must be repeated.

STORAGE

Store the opened / unopened product at 2-8°C until the expiry date detailed on the product label.

Failure to store the product at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing may result in accelerated loss of reagent activity.

SPECIMEN COLLECTION

No special preparation of the patient/donor is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique into tubes containing EDTA or CPD. The specimen should be tested as soon as possible following collection. Samples that cannot be tested within 24 hours of collection should be stored at 2-8°C. Testing should be carried out within 14 days of collection*. Specimens displaying gross haemolysis or microbial contamination should not be tested with this reagent. Failure to store the specimens in the correct conditions may result in false positive or false negative results.

* AABB Technical Manual 20th Edition, 2020.

MATERIALS PROVIDED

Product code TS Anti-Human Globulin is composed of polyclonal rabbit Anti-Human IgG and murine monoclonal IgM Anti-human C3d from the cell line BRIC-8 in a buffer solution containing macromolecular chemical potentiators. The reagent contains 0.1% (w/v) sodium azide and bovine material. The product is supplied filtered to 0.22 µm. The reagent has been optimised for use by the recommended techniques without further dilution or additions.

Contents:

- 10 x reagent vials for **REF** TS-10X10ML-B
- 1 x information sheet

MATERIALS REQUIRED BUT NOT PROVIDED

Indirect Antiglobulin Technique -Normal Ionic Strength Saline (NISS):

- Test tube
- Phosphate buffered saline
- Incubator 37°C
- Timer
- Centrifuge (1000 rcf)
- IgG sensitised red cells (Coombs control cells)

Indirect Antiglobulin Technique - Low Ionic Strength saline (LISS):

- Test tube
- Phosphate buffered saline
- Low Isotonic Strength saline
- Incubator 37°C
- Timer
- Centrifuge (1000 rcf)
- IgG sensitised cells (Coombs control cells)

Direct Antiglobulin Technique:

- Test tube
- Phosphate buffered saline
- Timer
- Centrifuge (1000 rcf)
- IgG sensitised cells (Coombs control cells)

RECOMMENDED TECHNIQUES

The use of automated cell washers must be validated by the user.

1. **INDIRECT ANTIGLOBULIN TECHNIQUE – Normal Ionic Strength Saline (NISS)**
 - 1.1 To a clearly labelled clean glass test tube add 2 drops (80-100 µl) of the test serum.
 - 1.2 Add one drop (40-50 µl) of a 3-5% suspension of test red cells which have been washed three times and resuspended in PBS.
 - 1.3 Mix thoroughly and incubate at 37°C for 30-60 minutes.
 - 1.4 Wash the cells four times in PBS taking care to decant the washing fluid completely and resuspending the cell button after each wash. Decant the PBS completely after the last wash.
 - 1.5 Add 2 drops (80-100 µl) of BIOSCOT® Anti-Human Globulin (polyspecific) to the dry cell button. Mix thoroughly and centrifuge at 1000 rcf for 20 seconds.
 - 1.6 Resuspend the cells by gentle agitation and read macroscopically. N.B.: vigorous agitation may disrupt weak agglutination.
 - 1.7 The validity of all negative antiglobulin tests should be confirmed by the addition of IgG sensitised red cells (Coombs control cells).
2. **INDIRECT ANTIGLOBULIN TECHNIQUE – Low Ionic Strength Saline (LISS)**

The use of LISS test cell suspensions enables the incubation time to be reduced to 15 minutes. The sensitivity of the LISS antiglobulin technique is dependent on the use of an equal ratio of serum to red cell suspension. It is therefore, recommended that semi-automated pipettes are used for the addition of serum and cell suspension. Test red cells should be washed twice in PBS and once in LISS before being adjusted to a 3-5% suspension in LISS.

 - 2.1 To a clearly labelled clean glass tube add 1 drop (40-50 µl) of test serum.
 - 2.2 Add an equal volume (40-50 µl) of 3-5% suspension of the test cells in LISS.
 - 2.3 Mix thoroughly and incubate at 37°C for 15 minutes. Continue through stages 1.4 – 1.7 as specified in the indirect antiglobulin technique (NISS).
3. **DIRECT ANTIGLOBULIN TECHNIQUE**

The direct antiglobulin technique is used to demonstrate in vivo adsorption of IgG and / or complement fragments to the red cells. The blood sample tested should be freshly drawn (less than 24 hours) and preferably collected into EDTA anticoagulant.

 - 3.1 Prepare a 3-5% suspension of test red cells in PBS.
 - 3.2 To a clearly labelled clean glass test tube add 1 drop (40-50 µl) of the cell suspension. Continue through stages 1.4 - 1.7 as specified in the indirect antiglobulin technique (NISS).

LIMITATIONS

Contamination with human serum and / or inadequate washing will neutralise Anti-Human Globulin.

False positive or false negative results may occur through contamination of test materials or any deviation from the recommended techniques.

PERFORMANCE CHARACTERISTICS

Anti-Human Globulin (cell line BRIC-8) polyspecific human IgG/C3d reagent product code TS has been tested by each of the recommended techniques with donor, clinical and neonatal specimens. The total number of tests (n) and the sensitivity and specificity was calculated for each technique and is displayed below:

Technique	Anti-Human Globulin Product Code TS			
	Sensitivity		Specificity	
	n	%	n	%
IAT (NISS)	0	0	51	100
IAT (LISS)	19	100	157	100
DAT	13	100	47	100

Abbreviations: IAT = Indirect Antiglobulin Test. DAT = Direct Antiglobulin Test. NISS = Normal Ionic Strength Saline. LISS = Low Ionic Strength Saline.

Diagnostic Sensitivity: The probability that the device gives a positive result in the presence of the target marker.

Diagnostic Specificity: The probability that the device gives a negative result in the absence of the target marker.

ANALYTICAL PERFORMANCE

This blood grouping reagent(s) exhibited unequivocal positive or negative results by all recommended techniques. Performance was found to be acceptable in terms of repeatability, reproducibility and robustness.

FURTHER INFORMATION

For technical assistance contact: SigmaAldrich.com/techservice

Any serious incident that has occurred in relation to this reagent must be reported to Millipore (UK) Ltd and the competent authority of the Member State in which the user and/or the patient is established.

The summary of safety and performance (SSP) for this device is available in the European database on medical devices (Eudamed) at <https://ec.europa.eu/tools/eudamed>, where it is linked to the Basic UDI-DI 4053252TSBTRVS

BIBLIOGRAPHY

1. Guidelines for the Blood Transfusion Services in the United Kingdom. 8th Edition 2013. The Stationary Office.
2. Issitt, P.D. and Anstee, D.J. Applied Blood Group Serology 4th Edition, Montgomery Scientific Publications, 1998.
3. AABB Technical Manual 20th Edition, 2020.

SUMMARY OF CHANGES

1. Rebranding & reorganisation of layout.
2. Materials provided- correct designation of rabbit Anti-Human IgG from monoclonal to polyclonal.
3. Identification of contents of packaging.
4. Update Intended Use section
5. Update specimen collection section
6. Clarification of drop volume in recommended techniques.
7. Update Intended Use section
8. Removal of clotted sample statement.
9. Removal of CTS definition
10. Addition of analytical performance section.
11. Addition of Further Information section.
12. Addition of technical service contact information.
13. Addition of requirement to contact Millipore (UK) Ltd and competent authority in the case of a serious incident involving this reagent.
14. Addition of information related to summary of safety and performance (SSP).
15. Removal of Introduction and References sections.
16. Addition of Bibliography section
17. Addition of Summary of Changes section.
18. Remove fax number.



Millipore (UK) Ltd
Fleming Road
Kirkton Campus
Livingston, EH54 7BN
United Kingdom
Tel: +44 (0)1506 404000

www.sigmaldrich.com

