

# Blood Group Monoclonal Reagent

Diagnostic reagent for the in-vitro qualitative detection of Blood Groups A, B, AB and D in Human Red Blood Cells manually.

|                            |                            |                             |
|----------------------------|----------------------------|-----------------------------|
| REF: BS.1/AA01.010.0010-3  | REF: BS.1/AA01.010.0010-5  | REF: BS.1/AA01.010.0010-10  |
| REF: BS.1/AB01.010.0010-3  | REF: BS.1/AB01.010.0010-5  | REF: BS.1/AB01.010.0010-10  |
| REF: BS.1/AD01.010.0010-3  | REF: BS.1/AD01.010.0010-5  | REF: BS.1/AD01.010.0010-10  |
| REF: BS.1/ABB01.010.0010-3 | REF: BS.1/ABB01.010.0010-5 | REF: BS.1/ABB01.010.0010-10 |
| REF: BS.1/AS03.010.0030    | REF: BS.1/AS04.010.0040    | REF: BS.1/AS10.010.0100     |

## CLINICAL SIGNIFICANCE

Red blood cells (RBCs) or erythrocytes are differentiated from each other on the basis of their surface antigen structures. It was Karl Landsteiner who first discovered the ABO blood group (BG) system in 1900 and rhesus (Rh) BG later.

the ABO and Rh BG system has fundamental importance in transfusions. For successful and safe blood transfusion, it is important to have knowledge about the compatibility of donor and recipient BGs, i.e., ABO and Rh. An incompatible or mismatched transfusion would make blood clump or agglutinate, which could lead to serious consequences and sudden death.

Four common phenotypes are now recognized: O, A, B and AB. Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity. Approximately 41% of the Caucasian population have the A antigen, 9% have the B antigen, 4% have both A and B antigens, while remaining have neither A nor B antigen.

The D antigen determinant is the most clinically significant non-ABO red blood cell antigen and the corresponding antibodies have been implicated in causing both Transfusion Reactions and Hemolytic Disease of the Newborn. Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or are derived from a human B cell line through EBV transformation. Approximately 85 % of the Caucasian population is Rh positive. The collective term Du is widely used to describe red cells which have a weaker expression of the D antigen determinant than normal.

**BioScien** reagent will detect most examples of partial and weak D red cells by direct agglutination in slide test and in tube technique.

## METHOD PRINCIPLE

The test principle is hemagglutination. The antibodies Anti-A, Anti-B and Anti-AB bind to the corresponding antigen on red blood cells being tested and cause an antigen-antibody reaction visible as red blood cell agglutination.

For all Anti-D negative test results should be further tested for Du (weak/partial D's) by performing the Du test procedure, as described later.

## REAGENT COMPOSITION

| Reagents:                                | Composition                                                                                                                       |
|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| <b>Monoclonal IgM ABO blood grouping</b> | Mouse monoclonal antibodies diluted in:<br>- Buffered protein solution containing bovine albumin<br>- EDTA<br>- Sodium azide 0.1% |
| <b>Anti-D Monoclonal</b>                 | Monoclonal blend of IgM and IgG antibodies diluted in:                                                                            |

|                    |                                                                         |
|--------------------|-------------------------------------------------------------------------|
|                    | - Phosphate buffer<br>- Bovine albumin<br>- Sodium azide 0.1%           |
| <b>Colored Dye</b> | - Anti-A<br>- Anti-B<br>- Anti-AB<br>- Anti-D                           |
|                    | - Blue Patent Blue<br>- Yellow Tartrazine<br>- Colorless<br>- colorless |

## PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagents contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

**S56:** dispose of this material and its container at hazardous or special waste collection point.

**S57:** use appropriate container to avoid environmental contamination.

**S61:** avoid release in environment.

For further information, refer to the Blood Group reagent material safety data sheet.

## REAGENT PREPARATION, STORAGE AND STABILITY

**BioScien** Blood Grouping reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

### Deterioration

The **BioScien** Blood Grouping reagent can be damaged due to microbial contamination or on exposure to extreme temperatures which is indicated by reagents turbidity. It is recommended that such reagent should be discarded.

## SPECIMEN COLLECTION AND PRESERVATION

Clean and dry glassware free from detergents must be used for sample collection, blood samples drawn with anticoagulant may be used for antigen determinant blood group typing. Samples should be stored at 2-8 °C if not tested immediately. Do not use haemolysed samples.

Anticoagulated blood using various anticoagulants should be tested

within the below mentioned time period:

- |                                    |           |
|------------------------------------|-----------|
| - EDTA or heparin                  | : 2 days  |
| - Sodium citrate or Sodium oxalate | : 14 days |
| - ACD or CPD                       | : 28 days |

## EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes (12x75 mm)
- Glass slides (50x75 mm)
- Centrifuge
- Stop watch
- Mixing sticks
- Pipettes (drop volume 40 to 50 µl)
- Isotonic saline solution

## ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature.

### Slide Test

1. Place on a labelled clean glass slide one volume (one drop) of **BioScien** Anti-A, Anti-B, Anti-AB and Anti-D reagents on specific place.
2. To each reagent drop add one volume (one drop approximately 50 µl) of whole blood.
3. Mix reagent and cells over an area of about 20x40mm. with separate mixing sticks.
4. Rotate the slide gently back and forth, for 30 seconds and observe for agglutination macroscopically at two minute.
5. Any weak reactions should be repeated by the tube technique.

### Tube Test

1. For Anti-A, Anti-B and Anti-AB prepare a 2-3% suspension of the washed test red cells in Isotonic saline. For Anti-D prepare a 5 % suspension.
2. Place one drop reagent Anti-A, Anti-B, Anti-AB and Anti-D reagents in an appropriately labeled test tubes.
3. Pipette into each of the test tubes, one drop (50µl) of the test red cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button, observing and read macroscopically for agglutination
6. Any tubes from Anti-A, Anti-B and Anti-AB, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Following incubation, repeat steps 4 and 5.

### D<sup>u</sup> Test Procedure (For Anti-D)

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of **BioScien** Anti-D reagent into labelled test tube.
3. Add to the test tube, one drop of the red cell suspension, mix well, and incubate at 37 °C for 15 minutes.
4. Wash the contents of the tube at least three times, with isotonic saline and decant completely after the last wash.
5. Add 2 drops of Anti Human Globulin reagent and mix well.
6. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
7. Resuspend the cell button gently and observe for agglutination macroscopically.

## STABILITY OF THE REACTIONS

1. Read all tube tests straight after centrifugation.
2. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
3. In Anti-D, complete washing steps without interruption and centrifuge and read tests immediately after reagent addition because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.

4. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

## READING AND INTERPRETATION

| Slide and Tube Technique |                                                                                                        |
|--------------------------|--------------------------------------------------------------------------------------------------------|
| Positive                 | Agglutination as indication for the presence of corresponding A and/or B and/or AB and/or D antigen.   |
| Negative                 | No agglutination as indication for the absence of corresponding A and/or B and/or AB and/or D antigen. |

## QUALITY CONTROL

The reactivity of all blood grouping reagents should be confirmed by testing with known positive and negative red blood cells to monitor the performance of the reagent. Good physiological saline may be used as a negative control, if the expected results have not been observed, the reagent should not be used. For more information please contact **BioScien** technical support.

## LIMITATIONS OF PROCEDURE

Some conditions that may cause false positive results are:

- Contamination of sample or reagents
- Autoantibodies
- Improper storage or preparation of red blood cells
- Antibodies to antibiotics or other reagents
- Cold Antibodies

## REFERENCES

1. Landsteiner, K. Ueber Agglutinationserscheinungen normalen menschlichen Blutes. Wien. Klin. Wochenschr. 1901, 14, 1132–1134.
2. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497.
3. 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.
4. Human Blood Groups by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995.
5. HMSO, Guidelines for the Blood Transfusion Services, 2nd Ed., 1994.

| SYMBOLS IN PRODUCT LABELLING                                                        |                             |                                                                                                                        |
|-------------------------------------------------------------------------------------|-----------------------------|------------------------------------------------------------------------------------------------------------------------|
|  | For in-vitro diagnostic use |  Number of <n> test in the pack   |
|  | Batch Code/Lot number       |  Caution                          |
|  | Catalogue Number            |  Do not use if package is damaged |
|  | Temperature Limitation      |  Consult Instruction for use      |
|  | Expiration Date             |                                                                                                                        |
|  | Manufactured by             |                                                                                                                        |