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BIOSCOT®

Anti-D

Monoclonal Human IgM/IgG
Blood Grouping Reagent

CE 0123 IVD

REF BM-10X10ML-B

Cell Line: TH-28/MS-26

Slide, Tube, Microplate & Indirect Antiglobulin, Gel and Column Techniques

INTENDED USE

BIOSCOT Anti-D (cell line TH-28/MS-26) monoclonal human IgM/ IgG blood grouping reagent is used to ensure the immunological compatibility of blood and blood components intended for transfusion. This qualitative reagent is used to detect the presence or absence of the D (RH1) antigen on the surface of human red blood cells when tested according to the slide, tube, microplate, indirect antiglobulin, gel and column techniques. The reagent is designed for in vitro diagnostic, professional use, by operators trained in serological techniques.

Weakened Expression of the RhD antigen

The term weak D denotes individuals with a reduced number of entire D antigen sites per red cell. The term partial D signifies individuals with missing D epitopes. D category VI (D^{VI}) is the partial D category which lacks the most D epitopes. Use of the indirect antiglobulin test with this reagent is required to detect some weak D phenotypes and D^{VI} cells. The slide and microplate methods are not recommended for the detection of weak or partial D cells. This reagent is ideal for patient and donor testing.

PRINCIPLE OF THE REAGENT

When used by the recommended techniques this reagent will cause agglutination (clumping) of red cells carrying the specific antigens (positive test). Lack of agglutination of the red cells demonstrates the absence of the specific antigen (negative test).

The reagent is intended for use by non-automated, manual techniques.

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

1. All blood products should be treated as potentially infectious. The human donor or the cell line used to produce this reagent has been tested and found to be negative for Anti-HIV, Anti-HCV, HBsAg, EBV and Mouse Antibody Production (MAP) viruses. No known tests can guarantee that any product derived from human blood is free from infectious agents. Care must be taken in the use and disposal of each container and its contents.
2. 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.
3. The product should be clear. Turbidity may indicate bacterial contamination. This reagent should not be used if a precipitate, fibrin gel or particles are present.
4. 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.
5. The product should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

CONTROLS

It is recommended that a positive and negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions.

It is not required to use a reagent control in parallel with all tests using this reagent. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control such as BIOSCOT Monoclonal control (Product code: TT) recommended. This should be tested in parallel with the reagent.

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.

MATERIALS PROVIDED

Product code BM Anti-D blood grouping reagent contains antibodies from cell lines TH-28 and MS-26. The reagent is composed of monoclonal human IgM and IgG antibodies 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 BM-10X10ML-B

1 x information sheet

MATERIALS REQUIRED BUT NOT PROVIDED

Slide Technique:
- Microscope slide
- Isotonic saline or compatible plasma/serum
- Timer

Tube Technique:
- Test tube
- Isotonic saline
- 37°C Incubator
- Timer
- Centrifuge (1000 rcf)

Microplate Technique:
- U well microplate
- Isotonic saline
- Timer
- Centrifuge (100 rcf)
- Microplate shaker
- Microplate reader (optional)

Indirect Antiglobulin Technique (for weakened expression of the D antigen)
- Test tube
- Anti-human globulin Reagent
- 37°C incubator
- Timer
- Centrifuge (1000 rcf)
- Isotonic saline
- IgG sensitised red cells (Coombs control cells)

MATERIALS REQUIRED BUT NOT PROVIDED (Continued

Ortho BioVue Column Technique:

- Ortho BioVue® AHG Polyspe-
- cific or AHG Anti-IgG Cassettes
- Isotonic saline, phosphate buffered saline (PBS) or Ortho® 0.8% Red Cell Diluent
- Micropipettes capable of
- delivering 10, 40 and 50 µL
- 37°C Incubator
- Timer
- Centrifuge suitable for Ortho BioVue Cassettes
- Reader (optional)

Bio-Rad ID Gel Technique:

- Bio-Rad ID-Card Coombs anti-IgG or LISS/Coombs
- Isotonic saline, phosphate buffered saline (PBS) or ID-Diluent 2
- Micropipettes capable of
- delivering 10, 25 and 50 µL
- 37°C Incubator
- Timer
- Centrifuge suitable for ID-Cards
- Reader (optional)

RECOMMENDED TECHNIQUES

1. SLIDE TECHNIQUE (Not recommended for the detection of weak or partial D phenotypes)

1.1 Prepare a 35-50% suspension of test red cells in autologous (or compatible) plasma, serum or in isotonic saline.
1.2 Add one drop (40-50 µl) of Anti-D reagent to a clean, labelled microscope slide.
1.3 Add one drop (40-50 µl) of the suspension of test red cells.
1.4 Mix the antiserum and cells over an area about 2 cm in diameter by gently and continuously rocking the slide.
1.5 Read macroscopically after 2 minutes. Do not confuse any drying of the mixture with agglutination.

2. TUBE TECHNIQUE

2.1 Prepare a 3-5% suspension of test red cells in isotonic saline.
2.2 Add 1 drop (40-50 µl) of Anti-D reagent to an appropriately labelled test tube.
2.3 Add 1 drop (40-50 µl) of the suspension of test red cells.
2.4 Mix and centrifuge at 1000 rcf for 20 seconds.
2.5 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
2.6 Negative or weakly positive tests may be tested by the indirect antiglobulin technique to detect weak or partial D phenotypes.

3. INDIRECT ANTIGLOBULIN TECHNIQUE (For Weakened Expression of the D Antigen)

3.1 Prepare a 3-5% suspension of red cells in isotonic saline.
3.2 Add one drop (40-50 µl) Anti-D reagent to an appropriately labelled test tube.
3.3 Add one drop (40-50 µl) of test red cells.
3.4 Mix and incubate at 37°C for 15 minutes.
3.5 Wash the cells once with isotonic saline thoroughly decanting the saline.
3.6 Add 2 drops (80-100 µl) of Anti-Human Globulin reagent, mix and centrifuge tests at 1000 rcf for 20 seconds.
3.7 Gently agitate the tube to dislodge the red cells. Examine macroscopically for agglutination.
3.8 To confirm that negative tests are valid, add IgG sensitised red cells (Coombs control cells), repeat the centrifugation, and examine for agglutination. If no agglutination is observed the test is invalid and should be repeated.

4. MICROPLATE TECHNIQUE (Not recommended for the detection of weak or partial D phenotypes)

4.1 Prepare a 3-5% suspension of test red cells in isotonic saline.
4.2 Add 1 drop (40 µl) of Anti-D reagent to the appropriate test wells of a U well microplate.
4.3 Add an equal volume (40 µl) of the test cell suspension to the appropriate test wells.
4.4 Mix the contents of each well using manual means or a microplate shaker.
4.5 Incubate the microplates at room temperature for 15-20 minutes.
4.6 Centrifuge the microplates at 100 rcf for 40 seconds.
4.7 Resuspend the red cells using the microplate shaker.
4.8 Read tests macroscopically or with a reader. The use of a plate reader must be validated by the customer.

5. BIO-RAD ID GEL TECHNIQUE

5.1 Prepare a 3-5% suspension of test red cells in isotonic saline/PBS or a 0.8% suspension in ID-Diluent 2.
5.2 Add 10 µL of 3-5% or 50 µL of 0.8% suspension of test red cells to the appropriate microtube of the ID-card.
5.3 Add 25 µL of Anti-D reagent to the appropriate microtube.
5.4 Mix gently and incubate at 37°C for 10 minutes.
5.5 Centrifuge the ID-card at the speed and time recommended in the ID-card manufacturer's instructions.
5.6 Read macroscopically or with a reader. The use of a reader must be validated by the user.

6. ORTHO BIOVUE COLUMN TECHNIQUE

6.1 Prepare a 3-5% suspension of test red cells in isotonic saline/PBS or a 0.8% suspension in Ortho 0.8% Red Cell Diluent.
6.2 Add 10 µL of 3-5% or 50 µL of 0.8% suspension of test red cells to the appropriate Ortho BioVue cassette reaction chamber.
6.3 Add 40 µL of Anti-D reagent to the appropriate reaction chamber.
6.4 Mix gently and incubate at 37°C for 10 minutes.
6.5 Centrifuge the cassette at the speed and time recommended in the cassette manufacturer's instructions.
6.6 Read macroscopically or with a reader. The use of a reader must be validated by the user.

LIMITATIONS

Red cells that have a positive direct antiglobulin test (DAT) may produce false positive results. The use of BIOSCOT Monoclonal Control Reagent (product code TT) is recommended for detection of such potentially false positive results.

Rigid polystyrene microplates are generally more suitable than those made from PVC. Each batch of microplates should be evaluated in the user's system prior to acceptance as suitable for routine usage.

Antigen variant cells may produce unexpected positive or negative reactions with samples previously typed with blood grouping reagents of polyclonal or other cell line-derived monoclonal sources.

Incorrect handling or storage of the ID-cards or Ortho BioVue cassettes could lead to incorrect results. Cards and cassettes should be stored and handled according to the manufacturer's instructions.

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

Slide and microplate techniques are not recommended for the detection of weak or partial D phenotypes.

PERFORMANCE CHARACTERISTICS

Anti-D (cell line TH-28/MS-26) monoclonal blood grouping reagent BM has been tested by each of the recommended techniques with donor, clinical and neonatal specimens. The sample population represented all major RhD phenotypes. The total number of tests (n), and the calculated sensitivity and specificity for each technique are displayed below.

TECHNIQUE	Anti-D Product Code BM			
	SENSITIVITY		SPECIFICITY	
	n	%	n	%
Slide	366	100	134	100
Tube	662	100	452	100
Microplate	366	100	134	100
Biorad ID Gel	282	100	214	98.2
Ortho BioVue	277	100	223	99.1

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 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-4053252BMBTRNA

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. Identification of contents of packaging.
3. Update Intended Use section
4. Removal of Introduction Section
5. Update specimen collection section
6. Clarification of drop volume in recommended techniques
7. Update Performance Characteristics Data
8. Remove Reference to Common Technical Specification (CTS)
9. Addition of Analytical Performance Section
10. Addition of Further Information section.
11. Addition of technical service contact information.
12. Addition of requirement to contact Millipore (UK) Ltd and competent authority in the case of a serious incident involving this reagent.
13. Addition of information related to summary of safety and performance (SSP).
14. Removal of References sections.
15. Addition of Bibliography section
16. Addition of Summary of Changes section
17. Remove fax number.

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