EN

INSTRUCTIONS FOR USE

Anti-D Blend

CE 0123

(IgM + IgG) monoclonal

REF 6785 1 x 10 ml

REF 6786 10 x 10 ml

Electronic Instructions for use see www.bag-diagnostics.com

FOR IN VITRO DIAGNOSTIC USE

1. Produkt Description

Anti-D Blend (IgM + IgG) monoclonal contains human monoclonal Anti-D (IgM), Clone D175-2, and human monoclonal Anti-D (IgG), Clone D415 1E4. Anti-D Blend (IgM + IgG) monoclonal is designed for use in slide and tube tests and provides a specific, qualitative test for the detection of the corresponding D (RH1) antigen on human red blood cells.
The diluent used for this low protein reagent contains NaCl, BSA, a buffer and other selected components to enhance the performance of the reagent. NaN_3 at a final concentration of 0.1% is added as a preservative.

Each lot Anti-D Blend (IgM + IgG) monoclonal has been tested according to methods recommended by the US FDA. The reagent meets the requirements of the Common Technical Specifications for products defined in Annex II, List A of Directive 98/79/EC on in vitro Diagnostic Medical Devices. When used in accordance with the recommended Instructions for Use the reagent has been tested and found to specifically agglutinate human red cells if the corresponding D (RH1) antigen is present.

The reactivity of each lot Anti-D Blend (IgM + IgG) monoclonal has been verified with a panel of red cells tested in accordance with the recommended Instructions for Use.

Anti-D Blend (IgM + IgG) monoclonal has demonstrated the ability to detect many cells of weak D phenotype by direct hemagglutination, which may previously have been interpreted as Rh negative (or weak D). This may include some types of unusual partial D cells that occur very rarely. The monoclonal IgM Anti-D clone D175-2 has not demonstrated reactivity with any D Category VI cell tested to date. The monoclonal IgG Anti-D clone D415 1E4 reacts with D Category VI.

The specificity of each lot has been verified by the recommended tube test method demonstrated with a panel of cells negative for the D (RH1) antigen. When suitable test cells are available, the presence of antibodies to low frequency antigens are excluded in routine specificity testing.

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2. Principle Of The Test

The test used with this blood grouping reagent is based on the principle of direct hemagglutination. Incubation of test red cells with Anti-D Blend (IgM + IgG) monoclonal will result in a specific antigen-antibody reaction if the corresponding D antigen (RH1) is present on the red cells. Visible detection of this reaction is demonstrated by agglutination of the cells. No agglutination indicates a negative test result, and within the accepted limitations of the test procedure, indicates the absence of the corresponding D (RH1) antigen on the test red cells.

3. Storage and Shelf Life

Store Anti-D Blend (IgM + IgG) monoclonal at 2...8°C. Do not freeze! Allow Anti-D Blend (IgM + IgG) monoclonal to reach room temperature (18...25°C) before use. Return reagent to 2...8°C for storage as appropriate, immediately after use.

Once Anti-D Blend (IgM + IgG) monoclonal has been opened the first time, the test reagent may be used up to the expiration date indicated on the label if the specified storage conditions are observed and no turbidity or other signs of contamination are observed. Do not use the reagent after the expiry date printed on the label. Do not use contaminated reagents!

4. Specimen Preparation

No special preparation of the patient/donor is required prior to specimen collection. Blood samples should be collected by approved aseptic medical procedures. Blood samples may be collected with or without anticoagulant if testing is performed without delay. If a delay in testing is unavoidable, red cells from clotted samples, or EDTA anticoagulated samples may be tested up to 14 days from date of collection. ACD, CPD and CPDA-1 anticoagulated blood samples may be tested up to their expiration date. All red cell samples should be stored appropriately at 2...8°C. A red cell preservative solution may be used for prolonged storage of red cells.

Prolonged storage of red cells prior to testing may result in deterioration of red cell antigens and resultant weaker than expected test reactions.

5. Additional Materials Required

Isotonic saline, pH 6.5 – 7.5

Glass slides and applicator sticks for mixing

Test tubes (75 x 12 mm, glass or polystyrene)

Single-use Pasteur pipettes

Centrifuge (900 – 1000 rcf)

Control red cells of known Rh phenotype

Anti-Human Globulin

IgG sensitized control cells (Coombs Control)

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6. Test Procedure

Slide Test Method

1. Prepare a 35 - 45% suspension of test red cells in isotonic saline.
2. Place 1 drop of test reagent on a labelled slide.
3. Add 1 or 2 drops of the prepared suspension of test red cells and mix thoroughly with a clean stick over an oval area of approximately 20 x 40 mm.
4. Slowly tilt the slide back and forth and examine for macroscopic hemagglutination within 2 minutes. Care should be taken not to mistake peripheral drying or fibrin strands as agglutination.
5. If the test is negative and a test for weak D is required, test according to the weak D Test Method.

Important notice for Rhesus-D-Determination on slides

The slide test method may not be sufficiently sensitive for reliable detection of weakened antigen expression and therefore not to be recommended. However, the slide test may be used for product control.

Do not place slides on heated surfaces.

Tube Test Method

1. Wash the test red cells with isotonic saline and prepare a 2 - 4% suspension of test red cells in isotonic saline.
2. Place 1 drop of test reagent and 1 drop of the prepared suspension of test red cells into a labelled test tube and mix thoroughly.
3. Centrifuge for 15 – 30 seconds at 900 - 1000 rcf or centrifugation of equivalent force.
4. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.

Please note:
Do not examine tests microscopically.

Weak reactions with Anti-D Blend (IgM + IgG) monoclonal may be enhanced following a 5 minute incubation at ambient room temperature (~18...25°C) and centrifugation as in steps 3 and 4 above.

Red blood cell suspensions known to be positive and negative for the D antigen should always be included in the test. For excluding spontaneous red cell aggregation, a control consisting of 6-8% BSA or autologous serum or plasma should be tested in parallel.

Use at least two different Anti-D test reagents to determine the D antigen. By use of two monoclonal test reagents two different clones should be used.

If the reaction is negative or doubtful the Modified Indirect Antiglobulin Test (Weak D Test) is required.

It is strongly recommended that the reactivity and specificity of the reagents be confirmed in regular intervals by control tests with known antigen positive and negative red cells (see statutory and local provisions). Positive cells should be selected to represent weak expression of the specific antigen and, when applicable, appropriate cells should be selected from heterozygous donors whose red cells express a single dose of the respective antigen.

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Modified Indirect Antiglobulin Test Method (Weak D Test Method)

1. Prepare a 2 - 4% suspension of washed test red cells. Red cells should be well washed at least once and resuspended in isotonic saline.
2. Place 1 drop of test reagent and 1 drop of the prepared suspension of test red cells into a labelled test tube and mix thoroughly.
3. Incubate the tube for 15 minutes at 37°C (± 1°C).
4. Wash the cells once with isotonic saline and decant the supernatant completely to ensure the removal of residual saline and a resultant “dry“ red cell button.
5. Add 2 drops of polyspecific Anti-Human Globulin or Anti-IgG to the “dry“ botton of cells (refer to the manufacturer`s Instructions for Use for Anti-Human Globulin) and mix gently but thoroughly.
6. Centrifuge without delay for 15 seconds at 900 - 1000 rcf or centrifugation of equivalent force.
7. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.
8. Confirm the validity of negative or only weak results with IgG sensitized control cells in accordance with the manufacturer`s Instructions for Use.

Please note:
Do not examine tests microscopically.
Red blood cell suspensions known to be D weak and known to be negative for the D antigen should always be included in the test.

Cells should not be used unwashed or suspended in plasma or serum for this test procedure.

7. Interpretation Of Test Results

Agglutination of test red cells with Anti-D Blend (IgM + IgG) monoclonal indicates the presence of the corresponding D (RH1) antigen within the accepted limitations of the test procedure. Very weak positive reactions may indicate the presence of quantitatively weak D or partial D antigen. Agglutination of test red cells with Anti-D Blend (IgM + IgG) monoclonal by weak D test procedure only (indirect antiglobulin test), indicates that the red cells are of the weak D phenotype. An Indirect Antiglobulin Test result with cells that demonstrate a positive Direct Antiglobulin Test cannot be reliably interpreted with respect to weak D.

No agglutination of test red cells with Anti-D Blend (IgM + IgG) monoclonal indicates the absence of the corresponding D (RH1) antigen within the accepted limitations of the test procedure.

When the controls react as followed, the test results cannot be interpreted:

- no agglutination with the test red cells known to be positive for the D antigen
- agglutination with the test red cells known to be negative for the D antigen
- agglutination with the BSA control
- agglutination with the patient control
- no agglutination with IgG sensitized control cells added to a negative Indirect Antiglobulin Test

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If different test results occur with two different test reagents, repeat the determination of the D antigen with a different test method and/or a different test reagent.

Pay attention to the limitations of procedure and important directions (s. 9. Important Directions / Limitations of procedure).

8. Stability Of The Reaction

All test results should be interpreted immediately upon completion of the test.

9. Important Directions/Limitations of Procedure

1. Anti-D Blend (IgM + IgG) monoclonal is designed for in vitro diagnostic use only and should be used by properly trained, qualified staff.
2. On rare occasion, red cells coated in vivo with immunoglobulin may agglutinate spontaneously and non-specifically in some reagent media. This phenomenon is usually associated with reagents formulated with high protein and macromolecular additives. Anti-D Blend (IgM + IgG) monoclonal is formulated in a low protein medium which does not promote spontaneous agglutination. However, very rarely, examples of red cells heavily coated with immunoglobulin may agglutinate non-specifically in this medium. In such instances similar phenomena would most likely occur in the ABO grouping test as well. If the test cells are reactive with anti-A, anti-B and anti-D, an additional control may be desirable. A control test consisting of either 6 - 8% BSA or patient serum/plasma may be suitable. If the control test yields a positive reaction, a valid interpretation of the Rh typing result cannot be made.
3. The use of unwashed test red cells suspended in plasma or serum may promote false positive reactions such as those associated with rouleaux formation or autoantibodies. The routine use of well washed, saline suspended red cells for tube tests may reduce the incidence of such false positive reactions.
4. Unwashed red cells or cells suspended in autologous serum or plasma must not be used in the Modified Indirect Antiglobulin Test for weak D detection as outlined herein; this could result in partial neutralization of the Anti-Human Globulin due to the abbreviated wash procedure and resultant weak or false negative results. If unwashed red cells are used, three to four sequential washing steps would be required to remove residual serum IgG sufficiently to perform an effective antiglobulin test.
5. Rhesus-D-determinations on slides are comparably less sensitive and therefore not to be recommended. However, the slide test may be used for product control.
6. A positive Indirect Antiglobulin Test for weak D must be validated by a macroscopically negative Direct Antiglobulin Test or a negative Indirect Antiglobulin Test using an appropriate control (i.e. 6 - 8% BSA).
7. Some red cells may express quantitatively weak and/or partial D (RH1) antigen and may therefore demonstrate weaker than expected reactions with Anti-D. Further clarification and specification of the result can be carried out with **BAGene** (BAG-SSP kits for the determination of Rh attributes on a molecular genetic basis).
8. Rare examples of red cells may express unusual forms of the D (RH1) antigen that lack specific epitopes (partial D). Anti D Blend (IgM + IgG) monoclonal will not detect all examples of partial D. In addition, this reagent may react with weak D cells and rare

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examples of partial D cells (i.e. R_0^{Har}, Crawford phenotype etc.) that may have previously been tested and interpreted as Rh negative using other sources of Anti-D.

9. The monoclonal IgM Anti-D clone D175-2 has not demonstrated reactivity with any D Category VI cell tested to date. The monoclonal IgG Anti-D clone D415 1E4 reacts with D Category VI.
10. Delays in reading tests, overvigorous resuspension of red cell buttons, and other technique variables associated with test performance may result in weaker than expected, or false negative test results.
11. Anti-D Blend (IgM + IgG) monoclonal must not be used to test enzyme treated red cells. Furthermore, to minimize other risks for false positive reactions, this reagent must not be tested when cold. Ensure that this reagent and any test cell samples are allowed to equilibrate to ambient room temperature (18...25°C) prior to testing.
12. False negative or unexpectedly weak reactions may occur with red cells that have been subjected to prolonged and/or inappropriate storage conditions.
13. Other variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware, incorrect saline pH and/or contaminated materials may cause false negative or false positive results.
14. The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended. No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.
15. Microbiological contamination of Anti-D Blend (IgM + IgG) monoclonal must be avoided as this may reduce the shelf life of the product and cause erroneous results. Do not use Anti-D Blend (IgM + IgG) monoclonal if marked turbidity or other observable indications of product alteration occur. These signs may indicate microbiological contamination and/or product deterioration.
16. Whether transfusions or transplantation have taken place should always be taken into consideration when interpreting the results. Any history of transfusions and/or transplantation, as well as the patient's medication history, should be taken into consideration when interpreting results.
17. The country-specific transfusion laws and/or directives (current laws or directives on transfusion medicine and blood group determination) must be taken into account.
18. Do not dilute Anti-D Blend (IgM + IgG) monoclonal. Use as supplied and as described in this Instructions for Use. Deviation from the recommended Instructions for Use may result in less than optimal product performance. Any deviation must be validated by the user.

10. Warnings and Precautions

Human source material used to produce this reagent has been tested and found negative for HBsAg and HIV and HCV antibodies. Nevertheless all used biological material should be handled as potentially infectious, because no test method can guarantee that products derived from biological sources are free from infectious agents. Any bovine albumin used in the manufacture of this product is sourced from donor animals that have been inspected and certified by veterinary service inspectors to be disease-free. This ruminant-based product is deemed to have low TSE (Transmissible Spongioform Encephalopathy) risk.

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When handling biological material appropriate safety precautions are recommended (do not pipette by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

The dropper bulbs of these products contain natural rubber latex, which is known to cause allergic reactions in some individuals.

The reagent contains 0.1% NaN_3. Sodium Azide is toxic. Do not ingest, avoid contact with the skin and mucous membranes. The copper and lead used in some plumbing systems can react with azides to form explosive salts. Therefore, when disposing of azide-containing materials, they should be flushed away with a large volume of water.

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

A Material Safety Data Sheets (MSDS) is available to download at www.bag-diagnostics.com.

11. References

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Explanation of symbols used on Labelling	
IVD	For in vitro diagnostic use
	Manufacturer
	Storage temperature / Temperature limitation
LOT	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
MONOCL | IGM + IGG	Monoclonal IgM + IgG
CLONE	Clone
ORIG | HUM	Origin: human
CONT | NaN_3	Contains Natriumazide
TIT	Titer
	**Warning** H302 Harmful if swallowed P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell P264 Wash hands thoroughly after handling P270 Do not eat, drink or smoke when using this product P281 Use personal protective equipment as required

Instructions for use	Version: 6/2019 / Issue: 2019-06

Instructions for use in other languages see:
http://www.bag-diagnostics.com
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