

INSTRUCTIONS FOR USE

Anti-D monoclonal (IgM)

CE 0123

saline / human

FOR IN VITRO DIAGNOSTIC USE

1. Product description

Anti-D monoclonal (IgM) is prepared from monoclonal, human Anti-D (IgM) (Clones: 597 Euclon and NaTH28-3c11) in isotonic saline. Anti-D monoclonal (IgM) is designed for use in tube and plate tests; and provides a specific, qualitative test for the detection of the corresponding D antigen on human red blood cells. This test reagent does not react with category VI. The test reagent contains < 0.1% NaN₃ as preservative.

The reactivity of each lot Anti-D monoclonal (IgM) is demonstrated by all given methods with several samples positive for the D antigen. The titer given on the label is determined by the tube test method with D positive red blood cells. The specificity of each lot is demonstrated by the recommended tube test method with a panel of red blood cells negative for the D antigen.

2. Biological principle of the test

The test used with this blood grouping reagent is based on the principle of hemagglutination. Incubation of test red cells with Anti-D monoclonal (IgM) will result in a specific antigen-antibody reaction if the corresponding D antigen is present on the test cells (Caution: Does not react with category VI !). 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 antigen.

3. Storage and Shelf Life

Store Anti-D monoclonal (IgM) at 2...8°C. Allow Anti-D monoclonal (IgM) to reach room temperature (18...25°C) before use. Return reagent to 2...8°C for storage as appropriate, immediately after use. After opening the bottle the test reagent can be used until the expiry date printed on the label, if appropriate storage conditions be observed. Do not use the reagent after the expiry date printed on the label.

4. Specimen preparation

Blood samples should be collected by approved medical procedure. Blood collected without or with anticoagulant (EDTA, heparin, citrate) is acceptable. Do not use haemolytic samples. Testing should be performed without delay if possible. Prolonged storage of red cells prior to testing may result in deterioration of red cell antigens and resultant weaker than expected test reactions (s. 9. Important Directions/Limitations of Procedure)

5. Additional Materials Required

Isotonic saline
Test plates for Blood Group Typing
Test tubes (75 x 12 mm)
Disposable Pasteur Pipettes
Centrifuge

6. Test procedure

Plate test

1. Prepare a 10% 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 on a test plate and mix.
3. Incubate for 30 minutes at room temperature.
4. By slowly rotation of the plate, examine macroscopically for agglutination.

Tube test

1. Prepare a 3 - 5% 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, mix and incubate for 5 minutes at room temperature.
3. Centrifuge for 1 minute at 150 x g (1000 rpm) or 20 seconds at 1000 x g (3000 rpm).
4. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.
5. If the reaction is negative, weak or doubtful, incubate the tube for 30 minutes at room temperature.
6. Centrifuge for 1 minute at 150 x g (1000 rpm) or 20 seconds at 1000 x g (3000 rpm).
7. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.

Directions: Do not examine tests microscopically.

Red blood cell suspensions known to be positive and negative for the D antigen, Rh-control for monoclonal test reagents and a patient control should always be included in the test.

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.

7. Interpretation of test results

Agglutination of test red cells with Anti-D monoclonal (IgM) indicates the presence of the corresponding D antigen (within the accepted limitations of the test procedure).

Caution: This test reagent does not react with category VI !

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

If no agglutination occurs with the test red cells known to be positive for the D antigen or if agglutination occurs with the test red cells known to be negative for the D antigen or the Rh control for monoclonal test reagents or with the patient control the test results should not be interpreted.

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

Pay attention to the limitations of procedure and important directions (s. 9. Important Directions/Limitation 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 monoclonal (IgM) 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 such instances similar phenomena would most likely occur in the ABO grouping test and blood grouping tests of other blood group systems as well. Rh control for monoclonal test reagents and patient autologous serum are suitable controls. 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 use of well washed red cells may reduce the incidence of such false positive reactions.
4. Some red cells may express quantitatively weak and/or partial Rh D antigen and may therefore demonstrate weaker than expected reactions or no reactions with Anti-D (s. 10. Performance characteristics). The plate test isn't suitable for the determination of weak and partial Rh D antigens. For the determination of weak and partial Rh D antigens it is recommendable to test with Anti-D Blend (IgM + IgG) additional; based on the IgG part it reacts often stronger. Further clarification and specification of the result can be carried out with **BAGene** (SSP-Kits for the determination of Rh attributes on a molecular genetic basis).
Anti-D monoclonal (IgM) does not react with category VI.
5. 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.
6. Anti-D monoclonal (IgM) should not be used for tests with enzyme treated red cells.
7. Haemolytic samples should not be used.
8. 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 sample are allowed to equilibrate to ambient room temperature prior to testing.
9. False negative or unexpectedly weak reactions may occur with red cells that have been subjected to prolonged and/or inappropriate storage conditions.
10. Other variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware, incorrect saline pH and/or contaminated materials and samples may cause false negative or false positive results.
11. Microbiological contamination of Anti-D monoclonal (IgM) must be avoided as this may reduce the life of the product and cause erroneous results. Do not use Anti-D monoclonal (IgM) if marked turbidity or other observable indications of product alteration occur. These signs may indicate microbiological contamination and/or product deterioration.
12. For interpretation of the test results, consider if transfusion or transplantation had happened. Take the case history of the transfusion or transplantation and also medicaments into consideration.
13. According to the German Guidelines for Collecting and Processing of Blood and Blood Components and for the Use of Blood Products from the scientific advisory board of the German Board of Physicians and the Paul-Ehrlich-Institut, German Federal Agency for Sera and Vaccines, blood donors are considered to be D positive if different test results occurs with different test reagents and if the test yields a weak positive reaction and blood recipients are considered to be D negative in case of different or questionable positive test results.

10. Performance characteristics

1012 samples of D positive (without D weak and D partial) and D negative blood donors, blood recipients and new-borns were tested in plate and tube test with BAG-Anti-D monoclonal (IgM) and a monoclonal Anti-D test reagent of an other manufacturer (s. table 1). All tests showed an agreement of 100% for the BAG test reagent with the comparable test reagent.

32 D weak and D partial samples were tested with BAG-Anti-D monoclonal (IgM) and a monoclonal Anti-D-test reagent of an other manufacturer (s. table 2). In tube test both test reagents reacted positive with D weak type I and III, D category VII, Rh 33, DNB and DBT. DFR erythrocytes reacted positive with the BAG test reagent and negative with the comparable test reagent in tube test. Both test reagents demonstrated no reactions or only very weak reactions (+/- reaction) with Category D V type VII and D weak type II erythrocytes in tube test.

The plate test proved to be not suitable for the determination of weak and partial Rh D antigens. Only one of the tested samples (D category VII) reacted positive with the both test reagents (BAG test reagent 2+ /3+ reaction; comparable test reagent 1+ reaction).

As expected both test reagents didn't react with D category VI.

Since due to the variety of rare D antigens all variants cannot be tested it doesn't have to be ruled out that Anti-D monoclonal (IgM) doesn't react with other not tested D variants.

Table 1	
Tested samples	1012
by that:	
D positive blood	767
D negative blood	245
EDTA blood	650
Heparin blood	120
Citrat blood	121
Blood of blood group A, B and AB	540
Blood donors	779
Clinical samples	181
Blood from new-borns	29

Table 2	
Tested samples (D weak and D partial)	32
by that:	
D weak / D partial, not further specified	16
D weak type I	2
D weak type II	2
D weak type III	1
D category V type VII	3
D category VI	1
D category VI type I	1
D category VI type II	1
D category VII	1
Rh 33	1
DNB	1
DBT	1
DFR	1

11. 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 material derived from biological sources are free from infectious agents. 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 test reagent contains NaN_3 as a preservative. The reagent contains $< 0.1\%$ NaN_3 which is not considered to be a harmful concentration. Nevertheless 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. The quantities of azide used in this reagent are small;

nevertheless when disposing of azide-containing materials, they should be flushed away with a large volume of water.

Disposal of all specimen and test materials should be in accordance with state and local law.











12. Packages: 1 x 10 ml, 10 x 10 ml, 25 x 10 ml, s. Catalogue

13. References

Applied Blood Group Serology, PD Issitt and DJ Anstee

4th Edition, Montgomery Scientific, Durham SC, 1998

Technical manual of the American Association of Blood Banks, 15th ed., 2005

Explanation of symbols used on Labelling	
	For in vitro diagnostic use
	Storage temperature
	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
	Monoclonal IgM
	Clone
	Origin: human
	Contains Natriumazide
	Titer

Instructions for use	Issue: August 2007
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