EN

INSTRUCTIONS FOR USE

Anti-Human-Globulin GREEN

CE 0123

(IgG + C3d) monoclonal

REF 6912 1 x 10 ml

REF 6913 10 x 10 ml

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

FOR IN VITRO DIAGNOSTIC USE

1. Description of product

The polyspecific Anti-Human-Globulin GREEN is prepared by blending cell culture supernatants produced by individual murine hybridoma cell lines. The final product contains material from two Anti-IgG cell lines (5H4 and 8D2-8), one Anti-C3c cell line (86 5A2) and one Anti-C3d cell line (139 4B4). This reagent is blended to react optimally with cells coated with IgG and/or C3 (C3b and/or C3d). The diluent used for this reagent contains sodium chloride, bovine serum albumin and selected buffers. Sodium azide, at a final concentration of 0.1%, is added as an antimicrobial agent. The polyspecific Anti-Human-Globulin GREEN contains Acid Blue #1 and Acid Yellow #23 as colouring agents.

Anti-Human-Globulin is used in a Direct Antiglobulin Test (DAT) and in an Indirect Anti-globulin Test (IAT).

A Direct Antiglobulin Test is used to detect antibodies and/or com-plement bound to red cells in vivo. Direct antiglobulin test results may support the diagnosis of Autoimmune Hemolytic Anemia (AIHA), Hemolytic Disease of the Fetus and Newborn (HDFN) and Delayed Hemolytic Transfusion Reactions (DHTR).

An Indirect Antiglobulin Test detects antibodies and/or complement bound to red cells in vitro. This technique is used in tests for weak D, crossmatch tests, detection and identification of unexpected blood group antibodies and red cell phenotyping.

Antiglobulin tests are also used by suspicion on drug induced red cell sensitization.

Anti-IgG, Anti-C3b and Anti-C3d potencies are assessed in serological tests with red cells coated specifically with IgG or C3 according to approved test procedures. Defined specificity is verified by tests against a variety of cells coated with different human proteins. The absence of contaminating heteroagglutinins is confirmed in serological tests against unsensitized cells of all ABO groups. The absence of detectable Anti-C4 is verified in serological tests against cells coated with C4b and C4d. This reagent reacts specifically with red cells coated with human IgG and/or C3 (C3b and C3d) when used in accordance with the recommended Instructions for Use.

2. Principle of the test

The Antiglobulin test detects human complement or human IgG bound in vivo or in vitro to red cells with the aid of anti-human globulins. The anti-human globulins contained in this reagent are monoclonal antibodies produced by hybridoma cells. The hybridoma cells derive from mice immunized with human complement or human immunoglobulins.
The anti-human globulins react with human complement and/or human immunoglobulins whether bound to the red cell membrane or present in the fluid phase. For the specific detection of red cell bound complement and/or immunoglobulins only, free serum immuno-globulins/complement must first be removed by a series of sequential wash procedures to ensure that only cell bound immunoglobulins/complement is present to react with the anti-human globulins. If red cells are coated with immunoglobulins/complement, anti-human globulins will bind specific to these proteins and forming bridges between the red cells. This reaction is optically recognizable by the agglutination of the red cells. If the red cells are not coated, no reaction occurs with the anti-human globulins and therefore no agglutination take place.

3. Storage and Shelf Life

Store Anti-Human-Globulin GREEN (IgG + C3d) monoclonal at 2...8°C. Do not freeze! Allow the reagent to equilibrate to ambient room temperature (18...25°C) prior to use. Return reagent to 2...8°C for storage as appropriate, immediately after use.
Once Anti-Human-Globulin GREEN (IgG + C3d) monoclonal has been opened the first time, the 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 medical procedures.

Direct Antiglobulin Test

To prevent significant in vitro fixation of complement, anticoagulated blood should be collected into EDTA, thereby allowing the specific detection of in vivo red cell complement sensitization. Other anticoagulants such as ACD or CPD may be less effective than EDTA, but remain acceptable for use. Blood samples should be tested as soon as possible following collection. If only clotted blood is available, it should not be refrigerated prior to direct antiglobulin testing (refer to Important Directions/Limitations of Procedure).

Indirect Antiglobulin Test

Serum samples should be prepared from freshly drawn clotted blood. Plasma should not be used if optimal detection of complement-binding red cell antibodies is desired, since complement activation by blood group antibodies may be in-hibited by the action of some anticoagulants that chelate Ca^{++}- and Mg^{++} ions. Active serum complement levels are depleted during storage, therefore optimal detection of complement-binding antibodies is achieved by testing serum from freshly drawn blood samples. Complement is degraded by 60% after 48 hours at room temperature but can be maintained at detectable levels for up to

BAG Diagnostics GmbH
Amtsgerichtsstr. 1-5
35423 Lich/Germany
Tel.: +49 (0) 6404 / 925 - 100
Fax: +49 (0) 6404 / 925 - 460
www.bag-diagnostics.com
info@bag-diagnostics.com

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Fax: +49 (0) 6404 / 925 - 421
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2 weeks at 4°C. A minimum of 60% normal complement activity is required to avoid missing weak complement binding antibodies. Alternatively, the serum may be frozen.
Plasma may be used if serum is unavailable and/or the test is performed solely to detect IgG sensitization.

5. Additional materials required

Isotonic saline, pH 6.5 – 7.5

Test tubes (75 x 12 mm), glass

Single-use Pasteur pipettes

Waterbath/Incubator (37°C ± 1°C)

Centrifuge (900 – 1000 rcf)

Reagent red blood cells with known phenotype for antibody detection and identification

Control cells sensitized with IgG (Coombs Control)

6. Test procedure

Direct Antiglobulin Test

1. Wash the test red cells at least once in isotonic saline and prepare a 2 - 5% red cell suspension in isotonic saline.
2. Add 1 - 2 drops of the washed, 2 - 5% red cell suspension to an appropriately labeled test tube.
3. Wash the red cells at least three times with large volumes of isotonic saline. Decant supernatant saline completely following each wash and ensure thorough resuspension and mixing of red cells with each new addition of saline for subsequent washes.
4. Following the final wash, completely decant the supernatant saline to ensure removal of all residual saline and a resultant „dry“ red cell botton.
5. Add 2 drops of Anti-Human-Globulin GREEN (IgG + C3d) monoclonal to each tube containing a “dry“ botton of washed red cells and mix gently, but thoroughly, to resuspend the red cells.
6. Centrifuge for 15 seconds at 900 – 1000 rcf or of equivalent force with adapted time.
7. Gently resuspend the red cell button and examine for agglutination.
8. Negative or weak positive antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells (refer to the relevant manufacturer’s Instructions for Use for IgG sensitized control cells).

Please note:
A microscope or other optical aid, may be used to confirm weak or negative hem-agglutination reactions.

The strength of anti-complement reactions may be enhanced following a 5 – 10 minute incubation at room temperature (~ 18...25°C) with subsequent re-centrifugation, as outlined above (steps 6 and 7). However, the immediate spin phase should never be omitted since anti-IgG reactions may be adversely affected by incubation and/or re-centrifugation.

As routine control the Anti-Human-Globulin should be tested against red cells weakly sensitized with IgG and against red cells coated with either C3b or C3d. Unsensitized red cells should be tested in parallel as a negative control.

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Indirect Antiglobulin Test

For Antibody Detection, Identification or Compatibility Testing

Please note:
The following test method is recommended only as a guide. Modifications to the test procedure following accepted and well documented immunohematology practices (such as an increase in serum:cell ratio and/or incubation time) may be desirable to comply with the requirements of individual laboratories. However, the application of low ionic strength antibody enhancement or potentiating reagents requires strict adherence to the respective manufacturer's recommended Instructions for Use. Red cell phenotyping with specific Blood Grouping Reagents should be performed in accordance with the manufacturer's Instructions for Use. User-defined modifications to test procedure may require validation.

The following is one example of a commonly used test protocol that may be used for antibody detection, antibody identification or compatibility testing:

1. Appropriately label one test tube for each donor cell, Screening Cell or Panel Cell to be tested.
2. Dispense at least 2 drops of serum and 1 drop of a 2 - 5% donor cell or reagent red cell (Screening Cell or Panel Cell) suspension into the appropriate test tube and mix thoroughly.
3. Centrifuge for 15 seconds at 900 – 1000 rcf or of equivalent force with adapted time.
4. Examine supernatant for visible hemolysis.
5. Gently resuspend the red cell button and examine macroscopically for agglutination.
6. Mix tube contents again and incubate test tubes at 37°C ± 1°C for 15 – 60 minutes.
7. Centrifuge for 15 seconds at 900 – 1000 rcf or of equivalent force with adapted time.
8. Examine supernatant for visible hemolysis.
9. Gently resuspend the red cell button and examine macroscopically for agglutination.
10. Mix tube contents gently, but thoroughly, and wash red cells 3 – 4 times with isotonic saline. Decant supernatant saline completely following each wash and ensure thorough resuspension and mixing of red cells with each new addition of saline for subsequent washes.
11. Following the final wash, completely decant the supernatant saline to ensure removal of all residual saline and a resultant "dry" red cell button.
12. Add 2 drops Anti-Human-Globulin GREEN (IgG + C3d) monoclonal to each tube containing a "dry" button of washed red cells and mix gently, but thoroughly, to resuspend red cells.
13. Centrifuge for 15 seconds at 900 – 1000 rcf or of equivalent force with adapted time.
14. Gently resuspend the red cell button and examine macroscopically for agglutination.
15. Negative or weak positive antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells (refer to the relevant manufacturer's Instructions for Use for IgG sensitized control cells).

Please note:
A microscope or other optical aid, may be used to confirm weak or negative hem-agglutination reactions.

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As routine control the Anti-Human-Globulin should be tested against red cells weakly sensitized with IgG and against red cells coated with either C3b or C3d. Unsensitized red cells should be tested in parallel as a negative control.

In other than low ionic test systems, it is a common and well-documented practice to increase the quantity of serum used in the test system (increase in serum:cell ratio) and/or to increase the incubation time beyond 15 minutes in order to increase the sensitivity of antibody detection/identification test procedures. When using low ionic enhancement techniques, however, the low ionic reagents must be used exactly as outlined in the respective Instructions for Use.

7. Interpretation of Test Results

Agglutination of test red cells in the antiglobulin test phase of the direct or indirect antiglobulin test procedure constitutes a **positive test result** and, within the accepted limitations of the test procedure, indicates the presence of IgG and/or complement (C3) on the red cells.

No agglutination of test red cells in the antiglobulin test phase of the direct or indirect antiglobulin test procedure constitutes a **negative test result** and, within the accepted limitations of the test procedure, indicates the absence of serological detectable IgG or complement (C3) on the red cells.

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

- no agglutination with IgG sensitized cells added to a negative antiglobulin test
- no agglutination with control red cells weakly sensitized with IgG
- no agglutination with control red cells coated with C3b or C3d
- agglutination with unsensitized red cells

The limitations of the procedure must be considered when interpreting the results (see 9. Important directions/limitations of procedure).

8. Stability of the reactions

All test results should be interpreted immediately following centrifugation.

9. Important Directions/Limitations of Procedure

1. Anti-Human-Globulin GREEN (IgG + C3d) monoclonal is designed for in vitro diagnostic use only and should be used by properly trained staff.
2. Positive direct antiglobulin test (DAT) results associated with complement sensitization may not reflect in vivo complement fixation if the test cells are from a previously refrigerated clotted blood sample.
3. Omission of the 5 – 10 minute incubation phase with subsequent re-centrifugation (for optimal detection of weak complement sensitization in the direct antiglobulin test (DAT) procedure) may result in weak or false negative results.
4. A negative direct antiglobulin (DAT) test result does not necessarily preclude clinical diagnosis of ABO Hemolytic Disease of the Fetus and Newborn (HDFN) or Autoimmune Hemolytic Anemia (AIHA).

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5. Red cells with a positive direct antiglobulin test (DAT) result cannot be used in indirect antiglobulin test procedures.
6. Certain disease states and medication therapy may be associated with positive direct and/or indirect antiglobulin tests.
7. Weak or false negative antiglobulin test results may occur due to inactivation by residual human serum protein following inadequate wash procedures or dilution of the Anti-Human-Globulin reagent associated with excess residual saline following wash procedures.
8. Procedural delays in antiglobulin test performance may result in weak or false negative results. Anti-IgG reactions, in particular, may be adversely affected by incubation and/or re-centrifugation.
9. Overly vigorous or inappropriate resuspension of red cells in antiglobulin test procedures may result in weak or false negative results.
10. To minimize risks for false results, this reagent must not be used when cold. Ensure that this reagent, specimen to be tested and control reagents are allowed to equilibrate to ambient room temperature (18...25°C) prior to testing.
11. False results may occur with specimen that have been subjected to prolonged and/or inappropriate storage conditions or if improper specimens are used.
12. 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.
13. 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.
14. Contamination of Anti-Human-Globulin with human serum may cause reagent neutralisation. Microbial contamination of Anti-Human-Globulin must be avoided as this may reduce the shelf life of the product and cause erroneous results. Do not use the reagent if marked turbidity or other observable indications of product alteration occur. These signs may indicate microbial contamination and/or product deterioration.
15. The use of green Anti-Human-Globulin does not preclude the necessity for adequate control and confirmation of antiglobulin reactivity. This dye provides a visual indication that the antiglobulin reagent has been added, but does not assure the reactivity of the reagent.
16. 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.
17. Do not dilute Anti-Human-Globulin GREEN (IgG + C3d) monoclonal. Use as supplied and as described in this Instructions for Use.
18. Deviation from the recommended Instructions for Use may result in less than optimal product performance. User-defined modifications to test procedures may require validation.

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10. Warnings and instructions for disposal

All materials of biological origin used for the test, especially the human specimen to be tested, should be regarded as potentially infectious. The absence of murine virus has not been determined. Any bovine source material 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.
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 this product contain natural rubber latex, which is known to cause allergic reactions in some individuals.

The reagent contains 0.1% NaN_3 as preservative. 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	Monoclonal
CLONE	Clone
ORIG | MURIN	Origin: mouse
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

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Instructions for use in other languages see:
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