BLOOD GROUPING REAGENT
NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend
For Slide, Tube and Microplate Test

IVD In Vitro Diagnostic Medical Device

Harmful – Contains 0.1% sodium azide
Components contain natural rubber latex

CE 0197

Consult Instructions for Use

Temperature Limitation - Store at 1-10°C.

EC REP IMMUCOR Medizinische Diagnostik GmbH
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AUTHORIZED REPRESENTATIVE IN THE EUROPEAN COMMUNITY

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	Use by (expiration)		Harmful
LOT	Batch code	REF	Catalogue number

Blood Grouping Reagent

NOVACLONE™

Anti-D IgM + IgG Monoclonal Blend

FOR SLIDE, TUBE AND MICROPLATE TEST

IMMUCOR

RECOMMENDED DIRECTIONS FOR USE

SUMMARY

The Rh_o (D) antigen was first recognized in 1939. Since the initial recognition of the D (RH1) antigen, over 50 different antigens are now known to be part of the Rh system. Most Rh blood group antibodies are immune, produced in response to stimulation by pregnancy or transfusion. The D (RH1) antigen is highly immunogenic and has been reported to stimulate the production of anti-D in 50-85% of D negative individuals who are exposed to D positive blood. Anti-D is of considerable importance since this antibody can cause severe Rh hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions. The D (RH1) antigen and its weakened form - weak D (formerly called D^u), are therefore important factors in the routine selection of blood for transfusion. Optimal detection of weak D cells by Anti-D Blood Grouping Reagents may require the application of an indirect antiglobulin test procedure. The commonly used terms Rh positive and Rh negative refer specifically to the presence or absence of the D (RH1) antigen. The frequency of Rh positive people in the Caucasian population is ~85%. More detailed information on the Rh system, its inheritance and nomenclature may be obtained from the references cited.

PRINCIPLE

The test used with this Blood Grouping Reagent is based on the principle of direct hemagglutination. Incubation of test red cells with NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend will result in a specific antigen-antibody reaction if the corresponding D (RH1) antigen is present on the red cells. Visible detection of this reaction is demonstrated by agglutination of the cells following centrifugation. Absence of 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.

REAGENT

FOR *IN VITRO* PROFESSIONAL DIAGNOSTIC USE ONLY

NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend contains human monoclonal IgM Anti-D (D175-2) and human monoclonal IgG Anti-D (D415 1E4). NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend is intended for use by slide, tube and microplate test 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 sodium chloride, bovine serum albumin, a buffer and other selected components to enhance the performance of the reagent. Sodium azide, at a final concentration of 0.1%, is used as an antimicrobial agent.
Do not dilute - Use as supplied.

PRECAUTIONS

Marked turbidity may indicate bacterial contamination or reagent deterioration. Do not use contaminated reagents or unlabeled vials. Do not use beyond expiration date. Store at 1-10°C when not in use. Do not freeze. Do not ingest.

Allow reagent to equilibrate to ambient room temperature (~18-25°C) prior to use.

SODIUM AZIDE IS TOXIC. DO NOT INGEST. SODIUM AZIDE MAY REACT WITH COPPER AND LEAD PLUMBING TO FORM EXPLOSIVE METAL AZIDES. ON DISPOSAL, FLUSH WITH LARGE VOLUMES OF WATER TO PREVENT AZIDE BUILD UP.

THIS PRODUCT HAS COMPONENTS (DROPPER BULBS) THAT CONTAIN NATURAL RUBBER LATEX, WHICH IS KNOWN TO CAUSE ALLERGIC REACTIONS IN SOME INDIVIDUALS.

ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. HUMAN SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

THIS PRODUCT SHOULD BE CONSIDERED BIOHAZARDOUS AND DISPOSAL SHOULD CONFORM TO APPLICABLE REQUIREMENTS FOR DISPOSAL OF BIOHAZARDOUS WASTE MATERIAL.

ANY BOVINE SOURCE MATERIALS, USED IN THE MANUFACTURE OF THIS PRODUCT, ARE 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 SPONGIFORM ENCEPHALOPATHY) RISK.

SPECIMEN COLLECTION

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. Red cells from clotted samples, or EDTA anticoagulated samples may be tested up to 14 days from collection[10]. 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 1-10°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.*
For Microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

PROCEDURES

Reagents Supplied: NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend (For Slide, Tube and Microplate Test).

Materials and Equipment Not Supplied: Transfer pipettes, isotonic saline (Phosphate buffered saline at a pH of 6.5-7.5 is recommended).
SLIDE TEST: Glass slides or plastic TP-12 plates, applicator sticks.
TUBE TEST: 12 x 75 mm or 10 x 75 mm glass or plastic (polystyrene) test tubes, test tube racks, serological centrifuge (900-1000 rcf).
MICROPLATE METHOD: Rigid U-bottom microplates, calibrated centrifuge with microplate carriers, microplate shaker (optional, but recommended).

Other Recommended Materials Not Supplied: Control red cells of known Rh phenotype; Anti-Human Globulin & IgG sensitized Antiglobulin Control Cells. NOVACLONE™ DILUENT CONTROL (OPTIONAL)

For Microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Users are responsible for validation of an accessory device for its intended use.

TEST PROCEDURES

Slide Test Method:

NOTE: Slide test procedures may not be sufficiently sensitive for reliable detection of weakened antigen expression.

Do not place slides/plates on heated surfaces.

1. Prepare a 35-45% suspension of test red cells. Red cell suspensions may be prepared in saline or autologous/group compatible serum or plasma (whole blood).
2. Add one drop of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend to one end of a labeled slide (or to one well of a TP-12 plate).
3. Using a transfer pipette, add one or two drops of the 35-45% suspension of test cells to each drop of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend.
4. Using clean, separate applicator sticks, thoroughly mix each red cell suspension over an oval area of approximately 20x40mm (or within each TP-12 microwell).
5. Slowly tilt the slide or plate back and forth for up to 2 minutes and examine for macroscopic hemagglutination.
6. At the end of 2 minutes, those tests showing no agglutination should be interpreted as negative. Care should be taken not to mistake peripheral drying or fibrin strands as agglutination.
7. If the test is negative and a test for weak D is required, test according to the weak D Test Method.

Tube Test Method:

1. Prepare a 2-4% suspension of test red cells in isotonic saline. (The routine use of washed red cell suspensions for blood grouping tests is recommended to reduce the risk of encountering anomalous reactions).
2. Dispense one drop of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend into an appropriately labeled test tube.
3. Using a transfer pipette, add one drop of the prepared 2-4% suspension of test red cells to the test tube.
4. Mix the contents of the test tube thoroughly.
5. Centrifuge for:
   a. 15-30 seconds at 900-1000 rcf.
   b. or centrifugation of equivalent force.

NOTE: 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.

6. Gently resuspend the red cell button and examine macroscopically for agglutination. **Do not examine microscopically.**
7. Grade and record results.

NOTE: Weak reactions with NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend may be enhanced following a 5 minute incubation at ambient room temperature (~18-25ºC) and centrifugation and resuspension as in steps 5 - 7 above.

Weak D Test Method - Modified Indirect Antiglobulin Test (IAT):
1. Prepare a 2-4% suspension of *washed* test red cells. For this modified IAT procedure test red cells must be *well washed at least once* and resuspended in isotonic saline.
2. Dispense one drop of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend into an appropriately labeled test tube.
3. Using a transfer pipette, add one drop of the prepared, washed 2-4% suspension of test red cells to the test tube.
4. Mix contents of the tube thoroughly and incubate at 37ºC (+/-1ºC) for 15 minutes.
5. Wash the cells *once* with isotonic saline.
6. Completely decant isotonic saline following the wash to ensure the removal of residual saline and a resultant "dry" red cell button.
7. Add 2 drops of Polyspecific Anti-Human Globulin or Anti-IgG to the "dry" button of cells in the test tube (Refer to the manufacturer's Directions for Use for Anti-Human Globulin).
8. Mix gently but thoroughly to resuspend the red cell button.
9. Centrifuge without delay for:
   a. 15 seconds at 900-1000 rcf.
   b. or centrifugation of equivalent force.
   c. or in accordance with the manufacturer's Directions for Use.
10. Gently resuspend the red cell button and examine macroscopically for agglutination. **Do not examine microscopically.**
11. Grade and record results.
12. Confirm the validity of negative tests using IgG sensitized Antiglobulin Control cells in accordance with the manufacturer's Directions for Use.

Note: This abbreviated antiglobulin wash procedure requires that the test red cells be pre-washed using isotonic saline, at least once, then resuspended in isotonic saline to a 2-4% concentration.
Cells should not be used unwashed or suspended in plasma or serum for this modified antiglobulin test procedure as described.

Microplate Method:
The following is a recommended manual method for microplate testing using this reagent. Alternate methods may be suitable if appropriately validated by the user.

NOTE: Microplates from different suppliers demonstrate variations in static properties, which may result in non-specific reactions of red cells and proteins. It is recommended that unused microplates be pre-treated, prior to use, to minimize red cell adherence.

Pre-Treatment of New, Unused Microplates:
1. To each microplate well, add one drop of 20-30% Bovine Serum Albumin (BSA).
2. Mix by gentle agitation or by using a microplate shaker to ensure the wells are evenly coated.
3. Allow the microplate to sit for 10-15 minutes at room temperature (~18-25º C).
4. Decant the BSA by flicking the microplate well contents into a suitable discard container.
5. Rinse the microplate at least 10 times with tap water.
6. Rinse the plate twice with distilled or deionized water.
7. Flick the plate and blot to remove excess water.
8. Allow the microplate to air dry prior to use.

Suggested Microplate Method:
NOTE: NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend is used in the following procedure without dilution or further modification.
1. Prepare a 2-4% suspension of red cells in isotonic saline. (The routine use of washed red cell suspensions for blood grouping tests is recommended to reduce the risk of encountering anomalous reactions).
2. Dispense one drop of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend to each microplate test well.
3. Add one drop of the 2-4% saline suspension of red cells to the appropriate test well.
4. Mix the contents of the wells thoroughly by manually tapping the microplate or, alternatively, by mixing on a microplate shaker†.
5. Centrifuge for 20-30 seconds at ~400g (350-450g) ‡.
6. Read and record the results using one of the following suggested methods.

Resuspension/Agitation Method:
1. Resuspend the red cell buttons in the wells by manually tapping the sides of the microplate or, alternatively, by using a microplate shaker¶.
2. Observe the microplate from the bottom and examine the wells for presence of agglutinates.

"Tilt and Stream" Method:
1. Tilt the microplate at an approximate 70° angle.
2. Allow 2-4 minutes for the cell buttons to start to disperse.
3. Observe the dispersion pattern of each well by viewing from the bottom of the microplate.

For Microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

NOTE: The use of supplementary visual aids such as a microplate test reading mirror or a hand lens may facilitate reading microplate tests.

† A *suggested mixing time for microplate shakers: 15-30 seconds at a medium setting.*

‡ *No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Each laboratory should calibrate their centrifuge equipment individually to determine the optimal centrifugation speed and time that produces the strongest agglutination reaction with antigen positive cells and allows complete and easy resuspension of negative reactions.*

¶ *A suggested resuspension guideline for microplate shakers is 30 seconds at a medium speed setting. Different microplate shakers vary in their orbit speeds, therefore each individual laboratory should calibrate their microplate shaker to determine the optimal speed and time required to achieve complete resuspension of negative test cells while maintaining maximum agglutination reaction strength with positive cells.*

CONTROLS
Appropriate control tests are essential for all laboratory test procedures.
1. False positive test results are rarely seen with low protein reagents. When observed, they usually indicate spontaneous red cell aggregation, which may occur even in saline. If desired, a Diluent Control for use with NOVACLONE™ Blood Grouping Reagents (NOVACLONE™ DILUENT CONTROL) may be tested in parallel. Alternately, a control consisting of 6-8% bovine serum albumin or autologous serum or plasma may be tested in parallel.
2. The application of IgG sensitized reagent control cells is considered an essential control procedure to confirm the validity of weak or negative antiglobulin tests.
3. It is strongly recommended that the reactivity of Blood Grouping Reagents be confirmed each day of use by control tests with antigen positive and negative red cells. 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.

For Microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

INTERPRETATION OF TEST RESULTS
Slide, Tube and Microplate Test:
POSITIVE (+): Within the accepted limitations of the test procedure, agglutination of test red cells with NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend indicates the presence of the corresponding D (RH1) antigen.
NOTE: Very weak positive reactions may indicate the presence of quantitatively weak D or partial D antigen. [Refer to Limitations of the Test Procedure following]

POSITIVE -Test for Weak D:
Within the accepted limitations of the test procedure, agglutination of test red cells with NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend by the weak D test procedure only (indirect antiglobulin test) indicates the test red cells are of the weak D phenotype.
Note: The failure of IgG sensitized Antiglobulin Control Cells to react when added to a negative IAT invalidates the original negative test result.
[Refer to Limitations of the Test Procedure following]

NEGATIVE (-): Within the accepted limitations of the test procedure, no agglutination of test red cells with NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend indicates the absence of the corresponding D (RH1) antigen.

NOTE: An Indirect Antiglobulin Test result with cells that demonstrate a positive Direct Antiglobulin Test cannot be reliably interpreted with respect to weak D – [Refer to Limitations of the Test Procedure following].

NOTE: If a patient control is run simultaneously with the test and shows agglutination, no valid conclusion concerning the test result can be reached.

Microplate Test:
Resuspension/Agitation Method:
A positive result is indicated by the presence of agglutinated cells that may be graded for reaction strength (similar to tube tests). Negative reactions are indicated by complete and smooth resuspension of red cells with no visible agglutinates.

"Tilt and Stream" Method:
Negative results are indicated by a smooth "streaming" of cells down the side of the microwell. A positive result is indicated by the presence of an intact button of cells remaining in the bottom of the well of the microplate. Alternatively, this button may become dislodged and fall in a large clump. Occasionally, positive reactions may appear as a solid monolayer of cells over the bottom of the well – such reactions usually appear as normal agglutination following resuspension or agitation.

Automated or semi-automated Microplate methods:
For interpretation of test results for microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

LIMITATIONS OF THE TEST PROCEDURE
1. On rare occasions, 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. NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend is formulated in a low protein medium, which does not normally promote spontaneous agglutination. Very rarely, however, examples of red cells heavily coated with immunoglobulin may agglutinate non-specifically in low protein media. In such instances, a similar occurrence would most likely be observed in the ABO grouping test - if the test cells are reactive with Anti-A and Anti-B and Anti-D, an additional control may be desired. A specific Diluent Control for use with NOVACLONE™ Blood Grouping Reagents (NOVACLONE™ DILUENT CONTROL) may be tested in parallel. Alternately, a control consisting of 6-8% bovine serum albumin or autologous serum or plasma may be suitable. If the control test yields a positive reaction, a valid interpretation of the Rh typing result cannot be made.
2. The use of unwashed test cells may promote false positive reactions such as those associated with rouleaux or autoantibodies. The routine use of washed, saline suspended red cells for tube tests may reduce the risk of such false positive reactions.
3. Unwashed red cells or cells suspended in autologous serum or plasma must not be used in the Modified Indirect Antiglobulin Test for Weak D 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 washes would be required to remove sufficient residual serum IgG to perform an effective antiglobulin test.

4. 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. NOVACLONE™ DILUENT CONTROL or 6-8% bovine serum albumin).
5. Some red cells may express quantitatively weak and/or partial D (RH1) antigen and may, therefore, demonstrate weaker than expected reactions with Anti-D Blood Grouping Reagents.
6. Rare examples of red cells may express unusual forms of the D (RH1) antigen that lack specific epitopes (partial D). NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend will not detect all examples of partial D. In addition, this reagent may react with weak D cells and rare examples of partial D cells (i.e. R_o^{Har}, Crawford phenotype etc.)[2]. that may previously have been tested and interpreted as Rh Negative using other sources of Anti-D.
7. Delays in reading tests, over vigorous resuspension of red cell buttons, and other technique variables associated with test performance may result in weaker than expected, or false negative test results.
8. NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend ***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.
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 or reagents may cause false negative or false positive results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Each lot of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend has been tested according to methods recommended by the US FDA. NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend 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 Directions for Use, NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend has been tested and found to specifically agglutinate human red cells if the corresponding D (RH1) antigen is present. The reactivity of each lot of NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend has been verified with a panel of red cells tested in accordance with the recommended Directions for Use. NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend has a demonstrated ability to detect many examples of weak D cells 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 component derived from cell line D175-2 has not demonstrated reactivity with any partial D Category VI cell tested to date. The specificity of each lot has been verified by the recommended tube and microplate test methods 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.

Deviation from the recommended Directions for Use may result in less than optimal product performance. Slide test procedures may not be sufficiently sensitive for reliable detection of weakened antigen expression.
User-defined modifications to test procedures may require validation.

REFERENCES

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PRODUCT:	ITEM CODE	
	1 x 10 mL	10 x 10 mL
NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend	5350012	5350022
NOVACLONE™ Anti-D IgM + IgG Monoclonal Blend (Galileo)	0066036	0066037

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