Anti-Human Globulin (Murine Monoclonal Blend)
NOVACLONE™ Anti-IgG, -C3d
Polyspecific (Green or Clear)

IVD In Vitro Diagnostic Medical Device

Harmful – Contains 0.1% sodium azide

Components contain natural rubber latex

Consult Instructions for Use

10°C Temperature Limitation – Store at 1-10°C

CE 0197

EC REP IMMUCOR Medizinische Diagnostik GmbH
Robert-Bosch-Strasse 32
63303 Dreieich, GERMANY
AUTHORIZED REPRESENTATIVE IN THE EUROPEAN COMMMUNITY

Manufacturer:
Dominion Biologicals Limited
5 Isnor Drive, Dartmouth, Nova Scotia CANADA B3B 1M1
Tel: 902-468-3992 Fax: 902-468-3599

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

Anti-Human Globulin

NOVACLONE™

Anti-IgG, -C3d

Polyspecific (Green or Clear)

Murine Monoclonal Blend

IMMUCOR

For in vitro Professional Diagnostic Use Only.

RECOMMENDED DIRECTIONS FOR USE

SUMMARY

Moreschi first described the principle of the antiglobulin test in 1908[1]. In 1945, Coombs, Mourant and Race first applied the principles of this test to blood group serology[2,3]. The antiglobulin test is a sensitive technique used to detect human immunoglobulin and/or complement components bound to human erythrocytes. Anti-human globulin is antibody produced by animals in response to deliberate immunization with purified human immunoglobulins or complement (primarily beta globulins). Anti-human globulin is used in a Direct Antiglobulin Test (DAT) to detect antibodies and/or complement 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 (IAT) detects immunoglobulin and/or complement bound to red cells *in vitro* and is the basis for a wide variety of immunohematology tests including: tests for weak D (D^u), donor unit crossmatch, detection and identification of unexpected blood group antibodies and red cell phenotyping.

The following table indicates the most appropriate applications of Polyspecific Anti-Human Globulin, Monospecific Anti-IgG and Monospecific Anti-C3d antiglobulin reagents available from Dominion Biologicals Limited.

Test Applications	Polyspecific (-IgG, -C3d)	Monospecific (-IgG)	Monospecific (-C3d)
Investigation/Diagnosis of:			
Hemolytic Disease of the Fetus and Newborn	YES	YES	
Autoimmune Hemolytic Anemia	YES	YES[a]	YES[a]
Transfusion Reactions	YES	YES[a]	YES[a]
Drug-Induced Red Cell Sensitization	YES	YES[a]	YES[a]
Identification of Cell Surface Coat:			
General	YES		
Specific		YES	YES
Compatibility Testing	YES	YES[b]	
Detection of Unexpected Antibodies:			
Donor Sera	YES	YES[b]	
Patient Sera	YES	YES[b]	
Detection of Red Cell Antigens:	YES	YES	
Identification of Unexpected Red Cell Antibodies:			
Serum/Plasma	YES	YES[b]	
Eluate	YES	YES	

NOTES:

a) This reagent should not be used as a sole antiglobulin reagent. Cells should be tested for the present of both IgG and C3d using either Polyspecific Anti-Human Globulin (-IgG, -C3d) or by using separate Anti-IgG and Anti-C3d in concurrent tests.

b) Some unexpected blood group antibodies have been reported to be better detected when the Anti-Human Globulin reagent contains anti-IgG and anti-complement. Current scientific evidence indicates that the exclusive use of monospecific Anti-IgG for these tests may, on rare occasion, result in failure to detect some blood group antibodies.

PRINCIPLE

The antiglobulin test is based on the principle of detection of erythrocyte-bound human complement or IgG, by heterophile antibodies, such as those produced in rabbits or by hybridomas derived from immunized mice. Anti-Human Globulin will react with human proteins whether bound to the red cell membrane or present in the fluid phase. For the specific detection of red cell bound proteins only, free serum globulins must first be removed by a series of sequential wash procedures to ensure that only cell bound globulin is present to react with the Anti-Human Globulin reagent. In this manner, Anti-Human Globulin will bind to sensitized red cells and mediate direct hemagglutination.

REAGENT

NOVACLONE™ Polyspecific Anti-Human Globulin 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. 0.1% sodium azide is added as an antimicrobial agent.

NOVACLONE™ Polyspecific Anti-Human Globulin (Green) contains Acid Blue #1 and Acid Yellow #23 as colouring agents. Do not dilute - Use as supplied.

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PRECAUTIONS

Marked turbidity may indicate bacterial contamination and/or reagent deterioration. Contamination of Anti-Human Globulin with human serum may cause reagent neutralization. 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 GROUPING REAGENTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. DO NOT INGEST. THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED.

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 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 may be 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 performing a DAT (refer to Limitations of Test 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 inhibited 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 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.

PROCEDURE

Reagents Supplied: NOVACLONE™ Polyspecific Anti-Human Globulin Anti-IgG, -C3d (Green or Clear)

Materials and Equipment Not Supplied:
Transfer pipettes, isotonic saline (phosphate buffered saline at a pH of 6.5-7.5 is recommended), 37°C (±1°C) incubator/waterbath, 12 x 75 mm or 10 x 75 mm disposable glass test tubes, test tube racks, timer, serological centrifuge (900-1000 rcf), reagent red blood cells for antibody detection/identification (IAT).

Other Recommended Materials Not Supplied: Low ionic strength antibody enhancement reagent (optional); IgG sensitized control cells, optical aid.

TEST METHOD

DIRECT ANTIGLOBULIN TEST PROCEDURE:

1. Wash an aliquot of test red cells at least once in isotonic saline and prepare a 2-5% red cell suspension in saline.
2. Add one to two drops of the washed, 2-5% red cell suspension to an appropriately labeled test tube.
3. Wash the red cells a minimum of 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 button.
5. Add two drops of NOVACLONE™ Polyspecific Anti-Human Globulin to each tube containing a ‘dry’ button of washed red cells.

6. Mix tube gently, but thoroughly, to resuspend the red cells.
7. Centrifuge for:
   a) 15 seconds at 900-1000 rcf.
   b) or centrifugation of equivalent force†.
8. Gently resuspend the red cell button and examine for agglutination.
9. Grade and record results. *(A microscope, or other optical aid, may be used to confirm weak or negative hemagglutination reactions).*
10. Negative or weak positive antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells (See CONTROLS).

*NOTE: 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 through 9). However, the immediate spin phase should never be omitted since Anti-IgG reactions may be adversely affected by incubation and/or re-centrifugation.

INDIRECT ANTIGLOBULIN TEST PROCEDURE
For Antibody Detection, Identification or Compatibility Testing

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 Directions for Use. Red cell phenotyping with specific Blood Grouping Reagents should be performed in accordance with the manufacturer's recommended Directions for Use. User-defined modifications to test procedures 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 two drops of serum into each tube‡.
3. Add one drop of 2-5% donor cell or reagent red cell (Screening Cell or Panel Cell) suspension to the appropriate test tube.
4. Mix tube contents thoroughly.
5. Centrifuge for:
   a) 15 seconds at 900-1000 rcf.
   b) or centrifugation of equivalent force†.
6. Examine supernatant for visible hemolysis.
7. Gently resuspend the red cell button and examine macroscopically for agglutination. Grade and record results.
8. Mix tube contents again and incubate test tubes at 37°C (±1°C) for 15-60 minutes.¶
9. Centrifuge for:
   a) 15 seconds at 900-1000 rcf.
   b) or centrifugation of equivalent force†.
10. Examine supernatant for visible hemolysis.
11. Gently resuspend the red cell button and examine macroscopically for agglutination. Grade and record results.
12. Gently, but thoroughly, mix tube contents again.
13. Wash red cells three to four 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.
14. Following the final wash, completely decant the supernatant saline to ensure removal of all residual saline and a resultant 'dry' red cell button.
15. Add two drops of NOVACLONE™ Polyspecific Anti-Human Globulin to each tube containing a 'dry' button of washed red cells.
16. Mix gently, but thoroughly, to resuspend red cells.
17. Centrifuge for:
   a) 15 seconds at 900-1000 rcf.
   b) or centrifugation of equivalent force†.
18. Gently resuspend the red cell button and examine for agglutination. Grade and record results. *(A microscope, or other optical aid, may be used to confirm weak or negative hemagglutination reactions)*.
19. Negative or weak positive antiglobulin test results should be appropriately controlled by the addition of IgG sensitized reagent control cells (See CONTROLS).

†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.

‡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). When using low ionic enhancement techniques, however, the low ionic reagents must be used exactly as outlined in the respective Directions for Use.

¶ In other than low ionic test systems, it is a common and well-documented practice to increase the incubation time beyond 15 minutes in order to increase the sensitivity of antibody detection/identification test procedures.

INTERPRETATION OF TEST RESULTS

POSITIVE: 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.

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

STABILITY OF THE REACTION:

All test results should be read and interpreted immediately following centrifugation.

CONTROLS
Appropriate control tests are essential for all laboratory test procedures.

1. The application of IgG sensitized reagent control cells to aid in the confirmation of effective antiglobulin test results is an essential control test for antibody detection/identification procedures which include an indirect antiglobulin test phase (Refer to the relevant manufacturer's Directions for Use for IgG sensitized control cells).
2. The specificity and reactivity of Anti-Human Globulin may be verified by routine quality control procedures. Polyspecific Anti-Human Globulin may be tested against red cells weakly sensitized with IgG and against red cells coated with either C3b or C3d to verify the presence of active Anti-IgG and Anti-C3 respectively. Unsensitized red cells should be tested in parallel as a negative control.

LIMITATIONS OF THE TEST PROCEDURE

1. Positive 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.
2. Omission of the 5-10 minute incubation phase with subsequent re-centrifugation (for optimal detection of weak complement sensitization in the DAT procedure) may result in weak or false negative results.
3. A negative DAT result does not necessarily preclude clinical diagnosis of ABO Hemolytic Disease of the Fetus and Newborn (HDFN) or Autoimmune Hemolytic Anemia (AIHA).
4. Red cells with a positive DAT cannot be used in indirect antiglobulin test procedures.
5. Certain disease states and medication therapy may be associated with positive direct and/or indirect antiglobulin tests.
6. 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.
7. Overly vigorous or inappropriate resuspension of red cells in antiglobulin test procedures may result in weak or false negative results.
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. 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.
10. 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.*

SPECIFIC PERFORMANCE CHARACTERISTICS

Each lot of NOVACLONE™ Polyspecific Anti-Human Globulin has been tested to ensure potency and specificity. 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 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 Directions for Use.

Deviation from the recommended Directions for Use may result in less than optimal product performance. User-defined modifications to test procedures may require validation.

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PRODUCT	PRODUCT CODES	
	10 mL vial	
NOVACLONE™ Anti-IgG; -C3d Polyspecific Murine Monoclonal Blend	CLEAR	5441
	GREEN	5451

Contact Customer Service at Dominion Biologicals Limited for ordering information.

Dominion Biologicals Limited
5 Isnor Drive
Dartmouth, Nova Scotia CANADA B3B 1M1
Tel: 902-468-3992; Fax: 902-468-3599

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