Anti-Human Globulin
Anti-IgG, -C3d; Polyspecific
(Rabbit/ Murine Monoclonal)
(BRIC 8)

FOR IN VITRO DIAGNOSTIC USE
For Tube Testing
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1845

Package size
REF 804115100 VOL 10 x 10 mL Anti-Human Globulin, Anti-IgG, -C3d; Polyspecific

Intended Use
Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is used for the direct antiglobulin test to demonstrate the in-vivo coating of red blood cells with antibody molecules and/or complement components (such as autoantibodies, maternal antibodies in Hemolytic Disease of the Newborn, alloantibodies against red blood cells in transfusion reactions).

Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is used for the indirect antiglobulin test to demonstrate the in-vitro coating of red blood cells with antibody molecules and/or complement components as in detection and identification of unexpected antibodies as well as crossmatch tests. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin-test) can be carried out.

Summary
Moreschi first described the use of Anti-Human Globulin in 1908. Coombs rediscovered the test in 1945. By injecting rabbits with human IgG, they were able to produce a protein (Anti-IgG) that reacted with "incomplete" antibodies (IgG). Most "incomplete" antibodies (IgG) fail to agglutinate red blood cells suspended in saline. Most clinically significant antibodies in red blood cell serology are of the IgG class and can only be detected by the use of Anti-IgG. A stable lattice structure is formed and agglutination occurs when Anti-IgG binds to the IgG sensitized red blood cells.

Bio-Rad Anti-Human Globulin reagents are used to test for the presence or absence of unexpected red blood cell antibodies. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin test) can be carried out. Routine pretransfusion studies always include tests for antibody screening, crossmatch and antibody identification.

Principle of the Test
The test principle is a hemagglutination test. Anti-Human Globulin Anti-IgG, -C3d; polyspecific acts as a link between the antibody and/or complement coating of neighbouring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.

Reagent
Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is a blend of rabbit anti-IgG and murine monoclonal anti-complement (murine IgM Anti-C3d, Bric 8). The anti-IgG component contains antibody reactivity against light IgG chains and thus may also agglutinate IgA or IgM coated red blood cells. The anti-complement component consists of murine monoclonal IgM anti-C3d-antibody reactive with C3b- and C3d-coated red blood cells.

Antibodies are diluted in an isotonic saline solution containing bovine albumin and as colorant Patent Blue and Tartrazine.

The following antibodies are produced using intermediate products produced for Bio-Rad Medical Diagnostics GmbH in a shared manufacturing agreement with Millipore (UK) Ltd., 9 Fleming Road, Kirkton Campus, EH547BN, Livingston, UK; License Number 1721.

Anti-C3d clone BRIC 8 (IgM)
Preservative: 0.1% Sodium azide.

Precautions
• For in vitro diagnostic use.
• Store at 2 to 8°C.
• Do not use beyond the expiration date.
• Do not use if turbid.
• Do not dilute.
• Do not use specimens collected with gel separators.
• Handle and dispose of reagents as potentially infectious.
• Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
• Caution: This Product Contains Natural Rubber Latex Which May Cause Allergic Reactions.
• Warning: Contains sodium azide (Na3N2), which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.

• The bovine albumin used for the production of this reagent is sourced from donor animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service inspectors to be disease free.

Specimen Collection
Fresh samples of clotted or EDTA anticoagulated whole blood can be used for the indirect antiglobulin test. EDTA or citrate anticoagulated whole blood samples must be used for the direct antiglobulin test. weak D test or crossmatch. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C. Serum or plasma may be separated from red blood cells and frozen. Stored samples should be allowed to reach room temperature prior to testing. Use of samples older than ten days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Blood specimens exhibiting gross hemolysis or contamination should not be used.

Materials
Materials provided
• Anti-Human Globulin Anti-IgG, -C3d; Polyspecific

Material required but not provided
• Pipettes ◄
• Isotonic saline
• Reagent red blood cells (e.g. Bio-Rad: Biotestcell Pool REF 816085100, Biotestcell® 1 & 2 REF 816014100, Biotestcell® 3 REF 816085100, Biotestcell®-I 11 REF 816021100, Biotestcell®-I 11 Plus REF 816022100)
• IgG coated red blood cells (e.g. Bio-Rad: Coombscell-E REF 816030100)
• Potentiators (e.g. MLB 2 Bio-Rad modified LISS REF 805200100 50 mL glas bottle or REF 805205100 10 x 10 mL glas bottle)
• Glass tubes 10 x 75mm or 12 x 75mm
• Serological centrifuge
• Interval timer
• Markers
• Agglutination viewer (optional). ◄

Test Procedure
A. Indirect Antiglobulin Test (IAT)
If an enhancement medium (albumin, LISS) is used, please refer to the respective instructions for use.

1. Prepare a 3 to 5% suspension of red blood cells in isotonic saline.
2. Place 1 drop (approx. 40 to 50 µL) of red blood cell suspension in an appropriately marked tube and add two drops of serum to be tested (or as directed for test reagent).
3. Incubate at 36 to 38°C for 30 to 60 minutes or as appropriate to the enhancement reagent used.
4. Wash the red blood cells 3 times with isotonic saline. Decant supernatant saline completely.
5. Add 2 drops of Anti-Human Globulin Anti-IgG, C3d; Polyspecific to the packed red blood cells and mix.
6. Centrifuge for:
   a. 20 seconds at 800 to 1000 x g or
   b. at a time and speed appropriate for the centrifuge calibration.
7. Gently dislodge the red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer. However, microscopic examination is not recommended.
8. Record results.
9. To control all negative antiglobulin tests, add red blood cells sensitized with IgG antibody, e.g. Coombscell E. (see package insert for procedure)

B. Direct Antiglobulin Test (DAT)
1. Prepare a 3 to 5 % suspension of the red blood cells in isotonic saline.
2. Wash 1 drop (approx. 40 to 50 µL) of this red blood cell suspension 3 times, with isotonic saline. Decant supernatant saline completely.
3. Add 2 drops of Anti-Human Globulin Anti-IgG, C3d; Polyspecific to the packed red blood cells and mix.
4. Centrifuge for:
   a. 20 seconds at 800 to 1000 x g or
   b. at a time and speed appropriate for the centrifuge calibration.
5. Gently dislodge the red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer. However, microscopic examination is not recommended.
6. Record results.
7. To control all negative antiglobulin tests, add red blood cells sensitized with IgG antibody, e.g. Coombscell E. (see package insert for procedure)

Stability of the Reaction
Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting in false negative or more often weak positive reactions.

Quality Control
The reactivity of all reagents should be confirmed by testing with known positive and negative red blood cells on each day of use. To confirm the reactivity or specificity of Bio-Rad Anti-Human Globulin Anti-IgG, -C3d; Polyspecific, the reagent should be tested with IgG coated (and if possible complement coated) and non coated red blood cells respectively. The reagent is satisfactory for use if it reacts only with the IgG (and complement) coated red blood cells.

Interpretation of Results
Agglutination of the red blood cells with the indirect antiglobulin test is a positive result and indicates the presence of an unexpected antibody(ies). Agglutination of the red blood cells with the direct antiglobulin test is a positive result and indicates an auto-agglutinin or auto antibodies. No agglutination is a negative result and indicates the absence of an unexpected antibody or the absence of the corresponding antigen or lack of an auto-agglutinin. An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual).

Limitations
- Low frequency antigens may not always be present on reagent red blood cells and a double dose of antigen may be required to detect very weakly reacting antibodies. Therefore, negative reactions with the screening red blood cells do not always indicate the absence of unexpected antibodies.
- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin Anti-IgG, -C3d; Polyspecific.
- Some conditions that may cause false positive results are:
   - Contamination of sample or reagents
   - Autoantibodies
   - Improper storage or preparation of red blood cells
   - Antibodies to antibiotics or other reagents
   - Cold antibodies
   - Positive reactions may be seen from individuals who have received Rh Immunoglobulin.
   - Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.
   - The performance characteristics with frozen/deglycerolized and enzyme treated red blood cells have not been established.

Specific Performance Characteristics
Testing is performed in accordance with FDA recommended methods. The final release testing is performed according to the product specific SOPs. As part of the release process each lot of Bio-Rad Anti-Human Globulin reagent is tested according to the package insert method against IgG and complement coated red blood cells to insure suitable reactivity. The product meets FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs. For the product performance it is necessary to adhere to the recommended method in the instructions for use.

The performance of the Bio-Rad Anti-Human Globulin Anti-IgG, -C3d; Polyspecific was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

For Technical Support or further product information, contact Bio-Rad Laboratories, Inc., at 800-224-6723.

Note
Manual techniques are to be performed according to the manufacturer’s instructions. Each deviation from these instructions is the sole responsibility of the user. Used tests must be discarded as hazardous material. Manage waste according to national regulations.

Glossary of Symbols

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<tr>
<th>Symbol</th>
<th>Definition</th>
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<th>Definition</th>
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<tbody>
<tr>
<td>[LOT]</td>
<td>Batch Code</td>
<td>[IVD]</td>
<td>In vitro diagnostic medical device</td>
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<td>Temperature limitation</td>
<td><img src="https://example.com" alt="🔍" /></td>
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Bibliography
1. Moreschi C. Neue Tatsache über die Blutkörperchen Agglutinationen, Zbl Bakter 1908; 46:49,456

Key: Underline = Addition of changes ❌ = Deletion of text

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