Anti-Human Globulin Anti-IgG (Rabbit) 

DOES NOT CONTAIN ANTIBODIES TO COMPLEMENT COMPONENTS

FOR IN VITRO DIAGNOSTIC USE

For Tube Testing

MEETS FDA POTENCY REQUIREMENTS

U.S. License Number: 1845

Package size

[REF] 804175100 VOL 10 x 10 mL Anti-Human Globulin Anti-IgG

Intended Use

Anti-Human Globulin Anti-IgG is used for the direct antiglobulin test to demonstrate the in-vitro coating of red blood cells with antibody molecules as in the 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 acts as a link between the antibody coating of neighbouring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.

Reagent

Anti-Human Globulin Anti-IgG is prepared by immunizing rabbits with human IgG. The anti-IgG component contains antibody reactivity against light chain IgG and thus may also agglutinate IgA or IgM sensitized red blood cells. There is no activity with complement coated red blood cells. The reagent is supplied in a 10 mL glass bottle.

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

Anti-Human Globulin Anti-IgG (Rabbit)

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 (NaN3), 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 the red blood cells and frozen. Stored samples should be allowed to reach room temperature prior to testing. Use of samples older than 10 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

Material required but not provided

- Pipettes
- Isotonic saline
- IgG coated red blood cells (e.g. Bio-Rad Coombscell-E [REF] 816030100)
- Potentiators (e.g. MLB 2 [REF] 805200100 50 mL glass bottle or [REF] 805205100 10 x 10 mL glass 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 reagent.
4. Wash the red blood cells 3 times with isotonic saline. Decant any enhancement reagent used.
5. An appropriately marked tube and add two drops of serum to be tested (or as directed for test reagent).
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. Coombs cell 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 one drop (approx. 40 to 50 µL) of this red blood cell suspension 3 times, with isotonic saline. Completely decant the supernatant.
3. Add 2 drops of Anti-Human Globulin Anti-IgG 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. Coombs cell 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 to 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, the reagent should be tested with IgG coated and non coated red blood cells respectively. The reagent is satisfactory for use if it reacts only with the IgG 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-aggulatin 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-aggulatin.

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 weakly reacting antibodies. Therefore, negative reactions with the screening 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.
- There is no anti-complement activity with this product. Cells coated with complement should not give a positive reaction.
- 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 Quality control by 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 was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

For Technical Support or further product information, contact BioRad 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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<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>LOT</td>
<td>Batch Code</td>
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<tr>
<td>!</td>
<td>In vitro diagnostic medical device</td>
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<tr>
<td>□</td>
<td>Caution, consult accompanying documents</td>
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<td>II</td>
<td>Consult instructions for use.</td>
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<td>☐</td>
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Bibliography

Key: **Underline** = Addition of changes  _Deletion of text_