ANTI-HUMAN GLOBULIN(AHG)

PRODUCT CODE:

BGAG0005, BGAG0010, BGAG1005, BGAG1010

QUALITY MANAGEMENT SYSTEM
ISO 13485 CERTIFIED COMPANY

BGAG0010	BGAG1010	BGAG1005
10ml	10 x 10ml	10 x 5ml
STORE AT 2-8°C		
INSTRUCTIONS FOR USE		
FOR IN-VITRO DIAGNOSTIC USE ONLY		

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

ANTI-HUMAN GLOBULIN (AHG)

POLYSPECIFIC – GREEN

For Direct & Indirect Antiglobulin Tests

Principle:
Polyspecific anti-human globulin reacts with human immunoglobulins and/or complement attached to the red cell surface, resulting in agglutination of the adjacent sensitised cells. In the antiglobulin test, red cells are washed thoroughly to remove globulins not bound to the red cell. Washing is necessary to prevent neutralisation of the anti-human globulin by unbound protein. Anti-IgG, -C3d polyspecific anti-human globulin added to the washed cells will cause agglutination if the cells are sensitised with immunoglobulin or complement. Unsensitised cells will not be agglutinated by anti-human globulin.

Presentation:

Reagent	Code	Size
Anti-Human Globulin - Green	BGAG0010	10 ml
Anti-Human Globulin - Green	BGAG1010	10 x 10 ml
Anti-Human Globulin - Green	BGAG1005	10 x 5 ml

Composition:
In order to produce the IgG portion of the Anti-Human Globulin, rabbits are immunised with purified human IgG. Any non-specific activity in the rabbit serum is removed by absorption. The anti-C3d component is an IgM antibody prepared from culture supernatants of a hybridoma derived from the spleen cells of an immunised mouse and a mouse myeloma cell. The rabbit anti-IgG and monoclonal anti-C3d are blended and diluted to optimal levels in a buffered solution containing bovine albumin.
The Fortress Polyspecific Anti-Human Globulin is green and contains Patent Blue V and Tartrazine.
The reagent contains 0.1 % sodium azide as a preservative and should be used as supplied.
Although all our components which have been derived from human origin have been tested and found to be negative for the presence of anti-HIV, anti-HCV as well as HbsAg, it is recommended that they be handled cautiously and treated potentially infectious.

Storage:
- Store components at 2-8°C.
- Do not freeze or expose to elevated temperatures.
- Do not use beyond the expiry date.
- Contamination with human serum or gamma globulin will inactivate anti-human globulin.
- Marked turbidity may indicate reagent contamination or deterioration.

Samples:
- For direct antiglobulin testing samples should be drawn into EDTA to prevent in vitro complement binding following collection and tested within 24 hours. If EDTA is unavailable, then specimens drawn into ACD, CPD or CPDA-1 are preferable to clotted specimens. If only clotted specimens are available, they should not be refrigerated prior to testing. Positive test results obtained with clotted specimens that have been refrigerated should be interpreted with caution.
- In indirect antiglobulin procedures for antibody detection or identification, fresh serum obtained from fully clotted specimens should be used. The use of plasma is not recommended since anticoagulants may interfere with the detection of complement-binding antibodies. Fibrin clots may also develop and interfere in tests employing plasma.
- Testing should be performed as soon as possible to minimise the chance that false positive or false negative reactions may be encountered due to contamination or improper storage.
- Sera that cannot be tested immediately should be stored at 2 - 8°C for no more than 48 hours.
- Frozen serum should be separated from red cells when stored.

Test Procedure:
The Fortress Anti-Human Globulin is standardised for use only in tube antiglobulin tests as described in the techniques set out below:-

1. **Direct Antiglobulin Test:**

The direct antiglobulin test is used to demonstrate in vivo sensitisation of cells. Washed red cells from a patient or donor are tested directly with anti-human globulin.

Tube Test:
1. Add one volume of a 2 - 3% suspension of the red cells to be tested to an appropriately labelled tube.
2. Using large volumes of Isotonic Buffered Saline with a pH of 6.9, wash the red cells at least four times, taking care to decant completely after each wash.
3. Add two volumes of Anti-Human Globulin to the dry cell button.
4. Mix the contents of the tube thoroughly.
5. Centrifuge the tube for 15 – 20 seconds at 900 – 1000 rcf.
6. Gently resuspend the cell button and examine for agglutination using either an optical aid or microscope. Record the results.
7. Confirm the validity of all the negative or weak positive results with IgG sensitised red cells.

Note: Room temperature incubation for up to 5 minutes and re-centrifugation may increase the strength of reactions due to the anti-complement components of this reagent. However, the initial centrifugation, following addition of anti-human globulin should not be omitted since some anti-IgG reactions may be adversely affected by additional incubation or re-centrifugation.

2. **Indirect Antiglobulin Test:**

The indirect antiglobulin test is used in compatibility testing, antibody detection, antibody identification, the D^{u} test or other tests for antigen detection. The test is used to demonstrate in vitro red cell sensitisation. Routine testing should incorporate a 37°C incubation phase followed by the antiglobulin phase. An autologous control (consisting of cells and serum from the specimen under test) should be tested in parallel when performing compatibility testing, antibody detection or antibody identification tests. Red blood cells with a positive direct antiglobulin test should not be used in tests for antigen detection by the indirect antiglobulin test method.

N.I.S.S. Tube Test:
1. Place in appropriately labelled test tubes two - four volumes of test serum and one volume of a 2 - 3% suspension of reagent red blood cells or cells prepared in isotonic buffered saline (pH 6.9). If bovine albumin or other potentiators are to be used, add and use according to the manufacturer's directions. Mix thoroughly.
2. Incubate at 37°C for 45 - 60 minutes.*
3. Using large volumes of Isotonic Buffered Saline with a pH of 6.9, wash the red cells at least four times, taking care to decant completely after each wash.
4. Add two volumes of Anti-Human Globulin to the dry cell button.
5. Mix the contents of the tube thoroughly.
6. Centrifuge the tube for 15 – 20 seconds at 900 – 1000 rcf.

7. Gently resuspend the cell button and examine for agglutination using either an optical aid or microscope. Record the results.
8. Confirm the validity of all the negative or weak positive results with IgG sensitised red cells.

* Depending on the potentiator employed, the tubes may be incubated for shorter periods of time. Consult the manufacturer's product insert for optimal incubation time for potentiating agent employed.

L.I.S.S. Tube Test:
1. Wash test cells and suitable positive and negative control cells twice in isotonic buffered saline (pH 6.9).
2. Wash cells once in L.I.S.S.
3. Resuspend washed cells to a 1.5% concentration in L.I.S.S.
4. Place in a prewarmed glass test tube:
5. 2 volumes of test serum
6. 2 volumes of 1.5% cell suspension.
7. Mix well and incubate at 37°C for 15 - 20 minutes.
8. Using large volumes of Isotonic Buffered Saline with a pH of 6.9, wash the red cells at least four times, taking care to decant completely following the final wash.
9. To the dry cell button add two volumes of Anti-Human Globulin.
10. Mix well and centrifuge for 15 - 20 seconds at 900-1000 rcf.
11. Gently resuspend the cell button and examine for agglutination. Reactions may be examined with an optical aid, or microscopically.
12. Confirm validity of all negative reactions by using IgG sensitised red cells.

Interpretation Of Results:
Agglutination of red blood cells in the presence of Anti-Human Globulin is a positive test result which indicates the presence of human IgG or components of complement on the red blood cells. A negative direct antiglobulin test does not necessarily rule out HDN, especially if ABO incompatibility is suspected. Haemolysis is not expected after the addition of anti-human globulin.

Reaction Stability:
Washing steps should be completed as soon as possible and without interruption. Following addition of the Anti-Human Globulin all tests should be centrifuged and read immediately. Delays in reading or delays in completion of washing steps may result in dissociation of antigen-antibody complexes leading to false negative or weak positive reactions.

Quality Control:
To confirm the specificity and reactivity of Anti-Human Globulin it is recommended that this reagent be tested each day of use with at least IgG sensitised red cells. This reagent can be considered satisfactory for use if it reacts suitably only with the sensitised cells. Anti-complement activity may be demonstrated by testing cells prepared by the two stage or low ionic strength sucrose techniques.

Limitations Of The Procedure:
False positive or negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper or excessive centrifugation, inadequate washing of red cells or introduction of human serum or gamma globulins, improper storage of test materials, or omission of test reagents.
Note: False negative indirect antiglobulin reactions have resulted from the use, for cell washing, of isotonic saline preparations sterilised in plastic bags designed primarily for irrigation and intravenous use. This problem has also been observed when solutions intended for cell counting and sizing have been used in place of saline in the antiglobulin test.

References:
Available on request.