ORTHO

Anti-Human Globulin Anti-IgG (Rabbit) (Green) *Ortho BioVue® System* (IgG Cassette)

INSTRUCTIONS FOR USE

REF

400 cassettes 707400
100 cassettes 707450

Intended Use

FOR *IN VITRO* DIAGNOSTIC USE
Qualitative procedure for the detection of IgG bound to red blood cells.

Summary and Explanation of the Test

Anti-IgG is an important diagnostic aid in determining the presence or absence of IgG on human red blood cells. This reagent will detect cells sensitized with IgG and will not react with cells sensitized with complement. Anti-IgG is a suitable reagent for direct and indirect antiglobulin testing.

Principles of the Procedure

The Ortho BioVue System utilizes column agglutination technology comprised of glass beads and reagent contained in a column which, upon centrifugation of the cassette, trap agglutinated red blood cells and allow nonagglutinated red blood cells to travel to the bottom of the column. Red cells are separated from serum proteins prior to exposure to the Anti-IgG reagent. The density of the reagent allows the red blood cells to pass through the column, while the less dense neutralizing serum proteins remain above the glass bead/reagent interface.

Reagents

Ortho BioVue System IgG cassettes are comprised of 6 columns containing a buffered solution with bovine albumin and macromolecular potentiators, as well as the preservatives 0.1% (w/v) sodium azide and 0.01M ethylenediaminetetraacetic acid (EDTA).

Product Codes 707400 and 707450	Component Description
Columns 1–6: Anti-Human Globulin, Anti-IgG (Rabbit) (Green)	Anti-IgG (rabbit) FD&C Blue No. 1 FD&C Yellow No. 5

Storage Requirement

- Store cassettes upright at 2–25 °C.
- **Do not store cassettes in a self-defrosting refrigerator/freezer.**
- **Do not store cassettes near any heat source (e.g., heat block, radiator, large instrumentation, refrigerator, freezer, etc., or any area receiving direct sunlight).**

Warnings and Precautions

DANGER:

This product contains 1-Imidazole (CAS 288-32-4) [1]

H360: May damage fertility or the unborn child. P280: Wear protective gloves, Eye protection. P308 + P313: If exposed or concerned: Get medical advice/attention.

Refer to www.Orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.

DANGER

1. Handle all blood and materials in contact with blood as if capable of transmitting infectious agents. It is recommended that blood and materials in contact with blood be handled using established good laboratory practices. [2]
2. All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. Some cassette components may be considered as hazardous or potentially infectious waste. Dispose of all materials according to applicable guidelines and regulations. [3]
4. Do not use reagents beyond their labeled expiration date.
5. Freezing of the cassettes or evaporation of the liquid due to heat may interfere with free passage of unagglutinated red blood cells through the glass bead column.
6. Do not use cassettes that appear damaged (i.e., break in foil seal or break, crack or bubble in the column) or exhibit drying (i.e., liquid level is at or below the top of the glass beads) or exhibit discoloration (due to bacterial contamination which can cause false reactions).
7. Use the Ortho BioVue System Centrifuge or ORTHO™ Workstation to provide the required centrifugation parameters for this system. Proper calibration of the centrifuge is essential to achieve accurate test results.
8. Erroneous results may be obtained due to improper technique in performing any diagnostic test. The most common sources of such results are:
   - Use of red blood cell concentrations other than those described under Specimen Collection and Preparation section
   - Microbial contamination of supplementary materials used in the procedure
   - Use of specimens containing particulate matter (impedes the free flow of red blood cells through the column)
   - Use of severely hemolyzed samples (may interfere with reading reactions in the column)
9. In order to minimize the presence of bubbles with your Ortho BioVue cassettes, we recommend that if you normally store your cassettes in the refrigerator at 2–8 °C you should equilibrate your cassettes at room temperature (20–25 °C) for at least 96 hours prior to use.
10. If testing is to be performed by methods other than those identified in the package insert, standardization of procedures must be undertaken to assure accuracy of test results.

Specimen Collection, Preparation and Storage

- No special preparation of the patient/donor is required prior to specimen collection.
- Blood should be collected by approved medical techniques.
- Samples should be tested as soon as possible following collection.
- If a delay in testing occurs, samples should be stored at 2–8 °C.

Note:	Red blood cells obtained from umbilical cord samples should be washed at least one time in isotonic saline.

DIRECT ANTIGLOBULIN TEST (DAT)

- Blood drawn into EDTA is preferred for the direct antiglobulin test.
- Blood drawn into heparin or with no anticoagulant (clotted specimens) should be tested within three days.
- Blood drawn into EDTA or sodium citrate should be tested within seven days.
- Donor blood may be tested up to the date of expiration.
- For the direct antiglobulin test, red blood cell suspensions can be prepared in the acceptable range of 3% to 5% using the following combinations of saline and packed red blood cells:

Saline Volume	Packed Red Blood Cell Volume*	Red Blood Cell Concentration
1 mL	40 µL	3%
1 mL	50 µL	4%
1 mL	65 µL	5%

* Blood samples centrifuged at 900 to 1000 x g for 5 minutes will result in a packed red blood cell concentration of approximately 80%. These specifications for centrifugation eliminate over-packing of red cells which may result in false-positive results. Data on file at Ortho-Clinical Diagnostics.

INDIRECT ANTIGLOBULIN TEST

- Either serum or plasma may be used when performing the indirect antiglobulin test in the Ortho BioVue System.
- This test is designed to be performed using a low ionic strength environment.
- Ortho 0.8% Reagent Red Blood Cells are supplied in a low ionic strength diluent suitable for this method.
- Red cell suspensions for crossmatch, autocontrol or antigen testing should be prepared using ORTHO® 0.8% Red Cell Diluent according to the instructions for use.

Note:	For LISS-additive methods, refer to ORTHO BLISS instructions for use.

Reagent Preparation

The Ortho BioVue System cassette is provided ready to use. Each column contains a single specificity of reagent suitable for one test. The cassette is heat-sealed with aluminum foil to preserve the integrity of the reagents. Upon opening of the foil seal, the cassettes should be used within one hour. Do not use the cassette if the liquid level in the column is at or below the top of the glass beads.

Reverse Diluent may be used as a control since it contains all components used in the IgG cassette except the rabbit anti-human IgG.

Procedure

Note:	Follow directions provided in commercially available antisera Instructions for Use when using this antisera.

The procedure identified below is for manual BioVue cassette testing only. When using automated instruments, follow the procedures that are contained in the operator's manual provided by the device manufacturer. Laboratories must follow their approved validation procedures to demonstrate compatibility of this product on automated systems.

Note:	For LISS-additive methods, refer to ORTHO BLISS instructions for use.

Materials Provided

- 400 cassettes (Product Code 707400)
- 100 cassettes (Product Code 707450)
- See Reagents section for component description

Materials Required but Not Provided

ORTHO™ Sera Blood Grouping Reagents

Direct Antiglobulin Test

1. ORTHO VISION® Analyzer
2. ORTHO VISION® Max Analyzer
3. ORTHO AutoVue® Innova / ORTHO AutoVue® Ultra Analyzers
4. Ortho BioVue System Centrifuge or ORTHO™ Workstation
5. ORTHO Optix™ Reader
6. Micropipetter for delivery of 10 µL
7. Disposable pipette tips
8. Ortho BioVue System Work Rack
9. Isotonic saline

Indirect Antiglobulin Test

1. ORTHO VISION® Analyzer
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3. ORTHO AutoVue® Innova / ORTHO AutoVue® Ultra Analyzers
4. Ortho BioVue System Centrifuge or ORTHO™ Workstation
5. ORTHO Optix™ Reader
6. Ortho BioVue System Heat Block, 37 °C
7. Micropipetter for delivery of 40 µL and 50 µL
8. Disposable pipette tips
9. Ortho BioVue System Work Rack
10. ORTHO 0.8% Reagent Red Blood Cells

11. ORTHO 0.8% Red Cell Diluent

Test Procedure for Direct Antiglobulin Test

1. Prepare red cell suspensions according to Specimen Collection, Preparation and Storage section.
2. Allow the cassette and test sample to come to room temperature before use. Orient the cassette with the back label (bar code side) facing you. Label the cassette appropriately with sample identification.
3. Peel off the foil strip on the top of the cassette only exposing the reaction chambers needed for the test(s) being performed. Visually inspect the cassette(s) to ensure that residual film does not block the opening of any wells after removing the foil.

Note:	The cassette should be used within one hour after removal of the foil. Cassettes with covered reaction chambers can be saved and these columns used for additional tests. Do not use the cassette if the liquid level is at or below the top of the glass beads.

4. Add 10 µL of 3% to 5% saline red blood cell suspension to the appropriate reaction chamber(s) of the cassette.
5. Centrifuge the cassette using the Ortho BioVue System Centrifuge or ORTHO™ Workstation.

Note:	Centrifugation should occur within 30 minutes of addition of the samples to the reaction chamber.

6. Read the front and back of the individual columns for agglutination upon test completion.
7. Record the reaction strength from the side with the stronger positive result.

Test Procedure for Indirect Antiglobulin Test

Note:	Follow directions provided in commercially available antisera Instructions for Use when using this antisera.

1. Prepare red cell suspensions according to Specimen Collection, Preparation and Storage section.
2. Allow the cassette and test sample to come to room temperature before use. Orient the cassette with the back label (bar code side) facing you. Label the cassette appropriately with sample identification.
3. Peel off the foil strip on the top of the cassette only exposing the reaction chambers needed for the test(s) being performed. Visually inspect the cassette(s) to ensure that residual film does not block the opening of any wells after removing the foil.

Note:	The cassette should be used within one hour after removal of the foil. Cassettes with covered reaction chambers can be saved and these columns used for additional tests. Do not use the cassette if the liquid level is at or below the top of the glass beads.

4. Add 50 µL of 0.8% red cell suspension to appropriate reaction chamber(s) of the cassette.
5. Add 40 µL of the patient's serum or plasma to the appropriate reaction chamber(s) of the cassette.

Caution:	**Do not touch the pipette tip to the side of the reaction chamber. If this occurs, change pipette tip before proceeding to the next chamber.**

6. Observe that the contents of the reaction chamber(s) are combined. If necessary, tap gently.

Note:	Assure that the reagents remain in the reaction chamber during incubation. There should be no mixing of reactants with reagents in the column prior to centrifugation. (See Limitations of the Procedure.)

7. Incubate at 37 °C for a minimum of 10 minutes to a maximum of 30 minutes.
8. Centrifuge the cassette using the Ortho BioVue System Centrifuge or ORTHO™ Workstation.

Note:	Centrifugation should occur within 30 minutes of addition of the samples to the reaction chamber.

9. Read the front and back of the individual columns for agglutination and/or hemolysis upon test completion.
10. Record the reaction strength from the side with the stronger positive result.

Quality Control Procedures

Serological testing is necessary to recognize reagent deterioration. It is recommended that each lot of reagents be tested on each day of use with appropriate positive and negative controls according to approved standard operating procedures.

Positive Control –	Use human serum or plasma containing an IgG antibody and antigen-positive red blood cells to test for the anti-IgG activity of the reagent. Follow the indirect antiglobulin test procedure. Follow the indirect antiglobulin test procedure.
Negative Control –	Use human serum or plasma free of unexpected red cell antibodies and unsensitized red blood cells. Follow the indirect antiglobulin test procedure.

Antiglobulin control cells are not required with the Ortho BioVue System and should not be used to control each individual test.

Interpretation of Results

Positive Result (+):	Agglutination of the red blood cells is a positive test result and indicates the presence of the corresponding antigen. The presence of hemolysis with or without agglutination is considered a positive test result.
Negative Result (-):	No agglutination or no hemolysis of the red blood cells is a negative test result and indicates the corresponding antigen is not demonstrable.

Hemolysis will result in a slight pink to red appearance in the reagent above the bead column. In cases of partial hemolysis, agglutination may or may not be present.

4+ Reaction	Agglutinated red blood cells form a band at the top of the bead column.
3+ Reaction	Most agglutinated red blood cells are retained or trapped in the upper half of the bead column.
2+ Reaction	Agglutinated red blood cells are observed throughout the length of the bead column. A small button of cells may also be visible at the bottom of the bead column.
1+ Reaction	Most agglutinated red blood cells are retained or trapped in the lower half of the bead column. A button of cells will also be visible at the bottom of the bead column.
0.5+ Reaction	Most agglutinated red blood cells pass through and form a disrupted (not smooth) button at the bottom of the bead column. Small agglutinates are visible above the button.
0 Negative	All red blood cells pass through and form a smooth button at the bottom of the bead column.

Limitations of the Procedure

1. The Test Procedure and Interpretation of Results must be followed closely to assure the accuracy of the test results. A laboratory that institutes the Ortho BioVue System should have a program that will train personnel on the proper use and handling of the product.
2. Some literature reports indicate that Anti-IgG may occasionally fail to detect antibodies that are demonstrable only by the use of an Anti-Human Globulin reagent containing anti-complement. Antibodies not detected by Anti-IgG may be clinically significant in some cases.
3. Low ionic strength solutions (LISS) have been shown to enhance many antigen-antibody reactions. However, samples may be encountered that contain antibody specificities, such as Anti-K (K1), which are not optimally reactive in LISS test systems.
4. Enzyme-treated red blood cells will nonspecifically agglutinate in the Anti-IgG reagent in the IgG cassette.
5. Abnormal serum/plasma proteins in the test sample may cause red blood cells to aggregate, which may be interpreted as agglutination.
6. Plasma expanders have been shown to interfere with some blood bank tests. Data are not available concerning interference using the Ortho BioVue System. Problem-solving techniques should be used if interference is observed.
7. Anomalous results may be caused by the following:

   - fibrin or particulate matter, such as clots, in serum or plasma
   - incompletely washed specimen red blood cells
   - mixing of reactants with reagents in the column (indirect antiglobulin test)
   - red blood cells that stick to the sides of the reaction chamber
   - bubble(s) that impede the passage of unagglutinated red blood cells

   An example of an anomalous result may be a line of red blood cells on top of the bead column. These may be prevented by removing fibrin or particulate matter from the sample by centrifugation before testing, washing red blood cells at least one time to remove residual plasma and/or platelets, and by reading results upon test completion. Tests with these or other anomalous results should be repeated.
8. When using automated instruments, refer to the limitations contained in the operator's manual provided by the device manufacturer.

Expected Results*

Antibody Detection (Indirect Antiglobulin Test)

Specificity analysis for the IgG cassette using 0.8% SURGISCREEN® Reagent Red Blood Cells was performed on the antibody screening results of 225 random population samples. The estimated specificity of 0.8% SURGISCREEN relative to 3% SURGISCREEN is 100% with a 95% confidence interval of 98.4% to 100.0%.

Sensitivity analysis for the IgG cassette using 0.8% SURGISCREEN was performed on the antibody screening results of 316 antibody-positive population samples. The distribution contained representative samples from the Rh-hr (32.59%), Kell (22.15%), Kidd (7.59%), Duffy (9.18%), MNS (7.59%), Lewis (6.33%) and P (1.27%) systems. Samples containing multiple antibodies (13.29%) were also included.

The estimated sensitivity of 0.8% SURGISCREEN relative to 3% SURGISCREEN is 100% with a 95% confidence interval from 98.8% to 100.0%.

Direct Antiglobulin Test (DAT)

In clinical studies using 3% to 5% red cell suspensions in saline with the IgG cassette, the results obtained for DAT by the BioVue method gave 99.6% (248/249) agreement when compared to the licensed tube test. There was 100% (237/237) agreement between tube and BioVue methods for direct antiglobulin testing of cord blood samples. Percent agreement indicates concordance between the two assays only and does not indicate which method gave the correct result.

*Data on file at Ortho-Clinical Diagnostics.

Specific Performance Characteristics[4-7]

The immunogen used to produce rabbit anti-human IgG is a gamma globulin fraction of human plasma. Testing for antibodies to immunoglobulin mu (μ) chains and light (κ and λ) chains is not performed but such antibodies may be present in this reagent.

Each lot of IgG cassettes is tested and shown to agglutinate red cells weakly sensitized with IgG. Unsensitized red cells and red cells sensitized *in vitro* with C3b and C4 are negative. This reagent may agglutinate IgM sensitized red cells.

Technical questions concerning these reagents should be directed to Ortho Care™ Technical Solutions Center.

References

1. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.
2. Laboratory biosafety manual. 2nd ed. World Health Organization, Avenue Appia 20, 1211 Geneva 27 Switzerland, 1993.
3. Biotechnology – Laboratories for Research, Development & Analysis – Guidelines for Handling, Inactivating and Testing of Waste. BS EN12740, BSI, 389 Cheswick High Road, London, W4 4AL, 1999.
4. Reis KJ, Chachowski R, Cupido A, Davies D, Jakway J, Setcavage TM. Column agglutination technology: the antiglobulin test. Transfusion 1993;33:639-43.
5. Vitali E, Musto L, Muffatti N, Serina S, Ciapponi E, Guardo P, Velati C. Comparison of two automatic methods for the detection of red cell alloantibodies (abstract). *Transfusion Clinique et Biologique* 2001;8 Suppl 1:170s.
6. Weisbach V, Ziener A, Zimmermann R, Glaser A, Zingsem J, Eckstein R. Comparison of the performance of four microtube column agglutination systems in the detection of red cell alloantibodies. *Transfusion* 1999;39:1045-50.
7. Mannessier L, Roubinet F. Detection of irregular antibodies by indirect antiglobulin test: comparison with polyspecific reagent and anti-IgG (abstract). *Vox Sang* 1998;74 Suppl 1:94.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Use by or Expiration Date (Year-Month-Day)	*In vitro* Diagnostic Medical Device	Fragile, Handle with Care.
LOT Batch Code or Lot Number	Temperature Limitation	Keep Dry
REF Catalog Number or Product Code	Consult instructions for use	This end up
Manufacturer	Cassettes	Do Not Use if Damaged
EC REP Authorized Representative in the European Community	Flammable	Keep away from Sunlight and Heat
Health Hazards	Acute Toxicity	Serious Health Hazards
Environmental or Aquatic Toxicity		Corrosive

Revision History

Date of Revision	Version	Description of Technical Changes*
2023-07-03	e631300076	Limitations of the Procedure: Added statement regarding Abnormal serum/plasma proteins

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

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EC REP

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