ORTHO

Blood Grouping Reagents
Anti-A (Anti-ABO1) (Monoclonal)
Anti-B (Anti-ABO2) (Monoclonal)
Anti-D (Anti-RH1) (Monoclonal)
Control
Reverse Diluent
Ortho BioVue® System
(ABO-Rh/Reverse Grouping Cassette)

INSTRUCTIONS FOR USE

REF

400 cassettes 707100
100 cassettes 707155

Intended Use

FOR *IN VITRO* DIAGNOSTIC USE

Qualitative test for the determination of the ABO blood group and D (RH1) antigens on human red blood cells and for determination of expected blood group antibodies.

Summary and Explanation of the Test

Testing with both Anti-A and Anti-B is necessary to determine if red blood cells possess or lack A (ABO1) and/or B (ABO2) blood group antigens. Normal adult individuals whose red cells lack A and/or B antigens usually have the corresponding antibody in their serum. The potentially serious consequences of ABO incompatible transfusions require that both transfusion recipient and donor red cells be reliably tested for the presence of A and B antigens. The results of ABO red cell grouping are confirmed by reverse (serum) grouping with known A_1 and B Reagent Red Blood Cells.

The D (RH1) antigen is capable of stimulating production of Anti-D in persons lacking the D antigen. Anti-D is a clinically significant antibody capable of causing red blood cell destruction and may result in hemolytic disease of the newborn (HDN) and transfusion reactions. The D antigen, therefore, is commonly considered in the routine selection of blood for transfusion and Anti-D immunoglobulin therapy.

Principles of the Procedure

The procedure used with these reagents is based on the principle of agglutination. Normal human red cells, possessing antigens, will agglutinate in the presence of antibody directed toward the antigen. The Ortho BioVue System utilizes column agglutination technology, comprised of glass beads and reagent contained in a column. Upon addition of red blood cells and subsequent centrifugation of the cassette, agglutinated red blood cells are trapped by the glass beads and nonagglutinated red blood cells travel to the bottom of the column.

Reagents

Ortho BioVue System ABO-Rh/Reverse Grouping 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 707100 and 707155	Component Description
Column 1: Blood Grouping Reagent Anti-A (Anti-ABO1)	Anti-A murine (IgM) monoclonal antibody blend (clones MHO4 and 3D3) FD&C Blue No. 1
Column 2: Blood Grouping Reagent Anti-B (Anti-ABO2)	Anti-B murine (IgM) monoclonal antibody blend (clones NB10.5A5 and NB1.19) FD&C Yellow No. 5
Column 3: Blood Grouping Reagent Anti-D (Anti-RH1)	Anti-D human (IgM) monoclonal antibody (clone D7B8)
Column 4: Control	Potentiator optimized for use as a control for blood group tests
Columns 5 and 6: Reverse Diluent	Potentiator optimized for use in reverse group test

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. Some cassette components may be considered as hazardous or potentially infectious waste. Dispose of all materials according to applicable guidelines and regulations. [3]

Caution:

Contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.

3. Improper storage conditions will adversely affect product performance.

Caution: **Attached to the cassette tray is a temperature monitor label. If the red showing through on the circle of the label meets or exceeds the color standard in the corner of the label, the cassettes have been exposed to temperatures above 42 °C, which can affect the performance of the reagents contained in the cassettes. Falsenegative results could occur with the use of these cassettes. Do not use the cassettes contained in the tray.**

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. Improper use of the liner assembly or dropping the cassette after the insertion of the liner could result in cross-contamination of reagents during pipetting.
9. 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, Preparation and Storage 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)

10. 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.

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 collected with anticoagulant or without anticoagulant may be used.
- Samples should be tested as soon as possible following collection.
- If a delay in testing occurs, samples should be stored at 2–8 °C.
- Clotted specimens or blood drawn into EDTA, heparin or sodium citrate should be tested within seven days.
- Donor blood may be tested up to the date of expiration.
- Red blood cells collected from the umbilical cord should be free from contamination (i.e., Wharton's jelly, tissue). If contamination is suspected, washing with isotonic saline may be necessary.
- Red blood cell suspensions can be prepared 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%
1 mL	10 µL	0.8%
0.8 mL	10 µL	1.0%

* 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.

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.

Procedure

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.

Materials Provided

- 400 cassettes (Product Code 707100)
- 100 cassettes (Product Code 707155)
- See Reagents section for component description

Materials Required but Not Provided

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, 40 µL and 50 µL
7. Disposable pipette tips
8. Ortho BioVue System Work Rack
9. Liner Assembly, BioVue
10. Isotonic saline
11. 0.8% OR 3% AFFIRMAGEN®

Test Procedure

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. Open the wells of the cassette using the liner assembly. Turn the cassette upside down and press down onto the liner. Slide the assembly out of the liner holder.

Note:	The cassette should be used within one hour after insertion of the liner.

4. For reverse grouping:
   - add 50 µL of 0.8% AFFIRMAGEN OR
   - 10 µL of 3% AFFIRMAGEN to the appropriate reaction chambers of the cassette (columns 5 and 6).
5. Add 40 µL of serum/plasma to the appropriate reaction chambers of the cassette (columns 5 and 6). If necessary, tap gently to mix contents of reaction chamber.
6. For forward grouping:
   - add 10 µL of a 3% to 5% OR
   - 40 µL of a 1.0% OR
   - 50 µL of a 0.8% red blood cell suspension to the reaction chambers of the cassette (columns 1, 2, 3 and 4).
7. 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.

8. Read the front and back of the individual columns for agglutination and/or hemolysis upon test completion.
9. 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 red blood cells known to possess the antigen toward which the reagent antibody is directed. If possible, a heterozygous expression of the antigen should be used. Results should demonstrate agglutination represented by red blood cells retained in or on top of the glass bead column.
Negative Control –	Use red blood cells known to lack the antigen toward which the reagent antibody is directed. Results should demonstrate no agglutination of the red blood cells represented by a button of packed cells at the bottom of the column.
Control Column –	Use normal (unsensitized) red blood cells. Results should demonstrate no agglutination of the red blood cells represented by a button of packed cells at the bottom of the column.
Reverse Diluent –	Test using standard procedures for reverse typing. Serum/plasma and reagent red blood cells should be selected to demonstrate both positive and negative reactions.

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 in the Reverse Group test 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.
Control Column:	If **any** degree of positive reactivity is observed in the Control Column, valid interpretation of the blood group **cannot** be determined. Further investigation by the user is required to determine the serological basis for the reactivity of the Control.

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.

Mixed cell populations may be detected by the Ortho BioVue System as agglutinated red blood cells at the top of the bead column and unagglutinated red blood cells at the bottom of the column. Detection limits may vary from those observed by other techniques.

Note:	Serum grouping and cell grouping should always agree. Discrepancies between the two should always be resolved. Refer to appropriate technical manual(s) for procedures used in resolution of ABO discrepancies.

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. Due to antigen deterioration, aged red blood cells may exhibit weaker reactivity than fresh cells.
3. Enzyme-treated red blood cells should not be used with these reagents.
4. In some patients (e.g., newborns, elderly or immunocompromised patients), the expected ABO antibodies may be weak or missing. For any recipient whose ABO group cannot be accurately determined, group O red blood cells should be considered as a transfusion alternative.
5. Red blood cells that appear to be D negative by this test method must be further tested for weak or partial D antigen by an acceptable test method when dictated by local requirements.
6. Good laboratory practice recommends that all weak Rh(D) positive typing results of 2+ or less be confirmed by an alternative method. [8, 9]
7. Invalid test results due to spontaneous agglutination may occur on rare occasions with the Anti-D reagent when testing red blood cells heavily coated with antibodies.
8. Abnormal serum proteins in the test sample may cause red blood cells to aggregate, which may be interpreted as agglutination.
9. 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.
10. High molecular weight polymers in the Reverse Diluent may enhance detection of red cell antibodies other than expected isoagglutinins.
11. When using automated instruments, refer to the limitations contained in the operator's manual provided by the device manufacturer.

Expected Results*

In clinical studies, the ABO and D (RH1) groupings for samples tested demonstrated the following distribution in the Ortho BioVue System:

Blood Group	Number of Samples Tested	Positive Samples	Frequency (%)
A (ABO1)	4253	1503	35.34
B (ABO2)	4253	524	12.32
AB (ABO3)	4253	203	4.77
O	4253	2023	47.57
D (RH1)	4231	3682	87.02

Ethnic backgrounds were available for 3264 (76.2%) of the samples tested. Of these samples, 61.6% were collected from persons of Caucasian background, 10.9% of African American background, 2.3% of Hispanic heritage, 0.9% of Oriental heritage, and 0.5% of American Indian, Saudi Arabian, Arabian, Asian Indian or Filipino heritage. Changes to the distribution will vary depending on the ethnic population under test.

The results obtained for ABO grouping by the BioVue method gave 99.95–99.98% agreement by forward testing and 99.70–99.78% agreement by reverse testing when compared to tube test. There was 99.93% agreement between tube test and BioVue methods for the detection of D (RH1) antigen. Percent agreement indicates concordance between two assays only and does not indicate which method gave the correct results.

*Data on file at Ortho-Clinical Diagnostics.

Specific Performance Characteristics*

Blood Grouping Reagents Anti-A (Monoclonal), Anti-B (Monoclonal) and Anti-D (Monoclonal), contained in the Ortho BioVue System cassette, have been tested and found to specifically agglutinate human red cells if the corresponding antigen is present, when used according to the recommended directions for use. [4, 5]

- The **Anti-A (Anti-ABO1) reagent** can detect most examples of the weak subgroups of the A antigen (such as A_2, A_3 and A_x) and may detect previously unrecognized A antigen in a small percentage of group B individuals referred to as B(A).[6] This reagent does not react with Tn polyagglutinable cells.
- The **Anti-B (Anti-ABO2) reagent** can detect some examples of the weak subgroups of the B antigen (such as B_3, B_x and B_m). This reagent does not react with acquired B antigen or Tn polyagglutinable cells.
- The **Anti-D (Anti-RH1) reagent** can detect most examples of weak and partial D (including weak D types 1, 2, 3 and 4.0 and D categories II, III, IV, V, VII, DBT and R_o^{Har}). It did not react with 9 of 9 D category VI cells tested.[7] Positive Rh(D) reactions of 2+ or less may indicate a weak D phenotype or spontaneous agglutination. Retesting with an alternative method will ensure the reactivity is due to the presence of the D-antigen and not due to spontaneous agglutination.[8, 9] This reagent may exhibit different serological activity when compared to other Anti-D reagents.

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

*Data on file at Ortho-Clinical Diagnostics.

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, Cupido A, Jakway J, et al. Red cell ABD/Rh/K typing by column agglutination technology. *Transfusion* 1992;32:Suppl 16S.
5. Reisner RK, Gauthier CM, Williamson KR, Moore SB. Comparison of patient ABO/Rh/K typing by column agglutination system and conventional tube method. *Transfusion* 1993;33:Suppl 18S.
6. Goldstein J, Lenny L, Davies D, Voak D. Further evidence for the presence of A antigen on group B erythrocytes through the use of specific exoglycosidases. *Vox Sang* 1989;57:142-6.
7. Reis KJ, Yaskanin D, Soto-Gil R, Ciavarella D. Evaluation of monoclonal anti-D reagents using clones D7B8 and RUM-1 in BioVue column agglutination test. *Vox Sang* 2000;78 (S1)P076.
8. Issitt PD, Anstee DJ. Weak D (formerly the Du Phenotype). In: Applied Blood Group Serology. 4th Ed. 1998;328-330:1185.
9. Thorpe SJ, Boult CE, Stevenson FK, Scott ML, Sutherland J, Spellerberg MB, Nativg JB ,Thompson KM. Cold agglutinin activity is common among human monoclonal IgM Rh sytem antibodies using the V4-34 heavy chain variable gene segment. *Transfusion* 1997;37:1111-1116.

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
Caution	Cassettes	Do Not Use if Damaged
Manufacturer		LATEX Contains or presence of natural rubber latex
EC REP Authorized Representative in the European Community		
Keep away from Sunlight and Heat		
Health Hazards	Flammable	Serious Health Hazards
Environmental or Aquatic Toxicity	Acute Toxicity	Corrosive

Revision History

Date of Revision	Version	Description of Technical Changes*
2024-02-13	e631300001	• Warnings and Precautions: Added Sodium Azide Caution statement • Glossary of Symbols: Added Caution symbol

* 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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