ORTHO

# Reverse Diluent
# *Ortho BioVue® System*
## (Reverse Diluent Cassette)

INSTRUCTIONS FOR USE



REF

**400 cassettes 707580**
**100 cassettes 707550**

## Intended Use

FOR *IN VITRO* DIAGNOSTIC USE

Principally for use in the determination of expected ABO blood group antibodies and antibody screen procedures for direct agglutination.

## Summary and Explanation of the Test

ABO blood type is determined by testing red blood cells with Anti-A, Anti-B and/or Anti-A,B reagents and by testing the serum/plasma for expected antibodies with a pool of known type $A_1$, $A_2$, B and/or O cells. In the Ortho BioVue System, the Reverse Diluent cassette is designed for use in detecting the presence of expected antibodies of the ABO system by direct agglutination. If group O cells are used with this system, they may detect the presence of saline (IgM) agglutinating antibodies.

## Principles of the Procedure

The procedures used with this reagent are based on the principle of agglutination. The Ortho BioVue System utilizes column agglutination technology, comprised of glass beads and reagent contained in a column. Antibodies present in normal human serum or plasma will agglutinate red blood cells that express corresponding antigens. Upon addition of serum or plasma and red blood cells to the reaction chamber of the column 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 Reverse Diluent 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 707580 and 707550 | Component Description |
|---|---|
| Columns 1–6: 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. Do not use reagents beyond their labeled expiration date.
4. 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.
5. 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).
6. 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.
7. 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)
8. 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.

## 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

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| **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.**

### Materials Provided

- 400 cassettes (Product Code 707580)
- 100 cassettes (Product Code 707550)
- 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. Isotonic saline
10. Reagent Red Blood Cells (Pooled Cells), 0.8% OR 3% AFFIRMAGEN®
11. 3% to 5% reagent red blood cells for antibody detection
12. ORTHO™ Sera Blood Grouping Reagents

### Test Procedure

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

1. 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.
2. Peel off the foil strip on 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.

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| Note: | The cassette should be used within one hour after removal of the foil. Do not use the cassette if the liquid level is at or below the top of the glass beads. |

3. For reverse grouping:
   - add 50 µL of 0.8% AFFIRMAGEN OR
   - 10 µL of 3% AFFIRMAGEN to the appropriate reaction chambers of the cassette
4. For antibody screening by direct agglutination (optional):
   Add 10 µL of 3% to 5% reagent red blood cells to the appropriate reaction chambers of the cassette
5. Add 40 µL of serum/plasma to the appropriate reaction chambers of the cassette.

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| **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. Centrifuge the cassette using the Ortho BioVue System Centrifuge or ORTHO™ Workstation.

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

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

### Quality Control Procedures

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|---|---|
| **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

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| 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. |

**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. 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. High molecular weight polymers in the Reverse Diluent may enhance detection of red cell antibodies other than expected isoagglutinins.
8. 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 reverse grouping for samples tested demonstrated the following distribution in the Ortho BioVue System:

| Cells | Number of Samples Tested | Positive Samples | Negative Samples |
|---|---|---|---|
| $A_1$ | 3651 | 2163 | 1488 |
| $A_2$ | 3651 | 2154 | 1506 |
| B | 3651 | 3034 | 617 |
| O | 3651 | 34 | 3617 |

The results obtained for reverse grouping by the BioVue method gave 99.70% agreement with $A_1$ cells, 99.45% agreement with $A_2$ cells, 99.78% agreement with B cells and 99.37% agreement with O cells when compared to tube test. Percent agreement indicates concordance between the two assays only and does not indicate which method gave the correct results.

*Data on file at Ortho-Clinical Diagnostics.

## Specific Performance Characteristics

The Reverse Diluent contained in the Ortho BioVue System cassettes has been tested and found to specifically agglutinate $A_1$, $A_2$ and B red cells if the corresponding antibody is present in the sample. [4, 5]

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. 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.
5. Pothiawala M, Musa G, Siwa C. Concordance of column agglutination technique to routine tube technique. *Transfusion*. 1993;33:Suppl 18S.

## 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 | e631300047 | • Warnings and Precautions: Added Sodium Azide Caution statement<br>• 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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