Blood Grouping Reagent
IH-Anti-D (RH1) Blend

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FOR IN VITRO DIAGNOSTIC USE
Anti-D reagent for use with IH-System
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1845

Product Identification: 77010

IH-Anti-D (RH1) Blend:	VOL 1 x 5 mL vial	REF 813 804 100

INTENDED USE

IH-Anti-D (RH1) Blend is intended to be used with the IH-Card AHG Anti-IgG to detect the D (RH1) antigen on human red blood cells, including weak D and partial D (DVI) by the indirect antiglobulin test using the IH-System.

SUMMARY

The Rhesus blood group system was first described by Landsteiner and Wiener in 1940. The antigen discovered by Landsteiner and Wiener is known as the ″D″ antigen. The terms ″Rh-positive″ and ″Rh-negative″ refer to the presence or absence of the D (RH1) red blood cell antigen. The D antigen is one of many that comprise the Rh blood group system. Approximately 85% of random donors in Caucasian populations have inherited the D gene and will phenotype as D positive.

The D antigen is probably the most important antigen outside of the ABO blood group system. Most D negative individuals will make anti-D when sensitized by the D antigen. Additionally, D negative females can become sensitized during pregnancy as a result of a fetal-maternal hemorrhage. The sensitization can lead to destruction of fetal red blood cells.

The D antigen is composed of many epitopes. Most of the D positive red blood cells have a complete protein. Weak D`s are defined by reduced amounts of the D antigen and can be classified in different types reflecting the number of D antigens on the red blood cells, which may require an indirect antiglobulin test for their detection. Partial D types are missing epitopes of the D antigen. This means that individuals possessing the DVI epitope may produce an anti-D to the missing epitopes after immunization by fetal or transfused D positive red blood cells

The IH-Anti-D (RH1) Blend is suitable for the detection of the D antigen including weak D and partial D (DVI). Please refer to the Specific Performance Characteristics section for additional information.

PRINCIPLES OF THE TEST

The test combines the principles of hemagglutination and gel filtration for detection of blood group antigen-antibody reactions.

The test sample (red blood cell suspension or plasma/serum) is distributed into the microtubes containing the appropriate reagent(s) and centrifuged. Non-agglutinated red blood cells are collected at the bottom of the microtube while the agglutinates are dispersed throughout the length of the gel, depending upon their size. Their position in the gel determines the intensity of the reaction. For the description of the reaction intensity, please refer to the Reaction Grading Guide in the Interpretation of Results section.

Please refer to the IH-Card AHG Anti-IgG instructions for use.

REAGENT

IVD
OBSERVABLE INDICATIONS
Do not use if markedly turbid**.**
NOTE: INSPECT THE CONDITION OF THE CARDS BEFORE USE (SEE PRECAUTIONS).

IH-Anti-D (RH1) Blend contains human monoclonal antibodies of the immunoglobulin classes IgM and IgG and is therefore suited for an indirect antiglobulin test to detect weak D and partial(DVI). The antibodies are derived from cell culture supernatant. This reagent contains bovine albumin that has been purchased from BSE-free sources.

IH-Anti-D (RH1) Blend cell lines BS232/BS221/H41 11B7 (IgM / IgG / IgG)
Ready-to-use reagent in a 5 mL vial

Preservative: Sodium Azide (0.1%)

STORAGE REQUIREMENTS

- Store at 2 to 8 °C.
- Do not use beyond the expiry on the label, which is expressed as YYYY-MM-DD (Year-Month-Day).
- Store in an upright position.
- Do not freeze or expose reagents to excessive heat.

• Do not store near any heat, air conditioning sources or ventilation outlets.

PRECAUTIONS

• All IH-System reagents and test samples must be brought to room temperature (18 to 25 °C) prior to use.
• Do not use reagent if showing marked turbidity
• Do not use cards showing signs of drying.
• Do not use cards with bubbles.
• Do not use cards with damaged foil strips.
• Use reagents as furnished.
• Caution: The packaging of this product (dropper bulbs) contains natural rubber latex which may cause allergic reactions.
• Warning: The IH-Card used with this reagent contains sodium azide, 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.
• Once the IH-Card has been used for testing, it may contain infectious material and should therefore be handled and disposed of as biohazardous waste in accordance with local, state, and national regulations.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Blood samples should be collected following general blood sampling guidelines.

Fresh blood samples collected in anticoagulants are acceptable. Samples should be tested as soon as possible after collection. If testing is delayed, EDTA samples may be stored at 2 to 8 °C for up to five (5) days or donor blood collected in CP2D may be tested up to thirty (30) days post collection when stored at 1 to 8 °C. Donor blood stored in additive solutions AS-3 may be tested up to thirty (30) days post collection when stored at 1 to 8 °C. Cord blood samples may be stored at 2 to 8 °C up to five (5) days post collection. Frozen and freshly thawed red blood cells are also acceptable for testing. Do not use grossly hemolyzed, lipemic or icteric samples.

Samples should be centrifuged for 10 minutes at 2000g or at a time and speed that consistently produces a distinct cell/plasma interface. Donor segments do not require centrifugation.

Please refer to the IH-Card AHG Anti-IgG instructions for use and the **IH**-1000 User Manual NA for sample requirements.

TEST PROCEDURE FOR AUTOMATED SYSTEMS

Material provided
• IH-Anti-D (RH1) Blend, 5 ml vial

Materials required but not provided
• IH-Card AHG Anti-IgG
• IH-LISS Rack

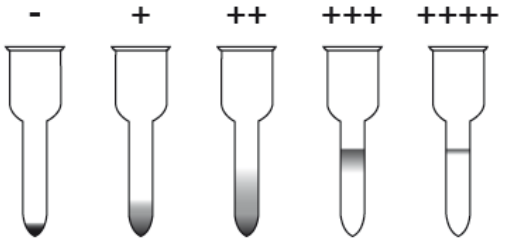
• **IH**-1000

Method
Please refer to the IH-Card AHG Anti-IgG instructions for use and the **IH**-1000 User Manual NA for testing and reagent handling instructions.

INTERPRETATION OF RESULTS

For automated systems

Below is a description of the various reaction grades and how the software uses that well reaction to determine the result interpretation. Please refer to the IH-Card AHG Anti-IgG instructions for use, and the **IH**-1000 User Manual NA for further information.



Well Reaction Grade	Result Interpretation	Reaction Description
-	Negative	A compact, pellet of RBCs* with a smooth surface at the bottom of the well with no visible agglutination.
+/-	Blood Grouping, Antisera, and Phenotyping including Anti-D Blend, = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive	A pellet of RBCs at the bottom of the well with a very few agglutinated RBCs visible above the pellet or an irregular pellet.

Well Reaction Grade	Result Interpretation	Reaction Description
	Antibody Identification= no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible	
1+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with agglutinated RBCs visible in the lower half of the gel column.
2+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs distributed throughout the entire length of the gel column, with no line of RBCs on the top of the well.
3+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Most agglutinated RBCs concentrated at the top of the gel or upper half of the gel column.
4+	For Blood Grouping, Antisera and Phenotyping including Anti-D Blend =Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible	Agglutinated RBCs concentrated as a line on the top of the gel column with a few agglutinated RBCs just underneath the gel surface.
Mixed Field (DP)	Blood Grouping, Antisera, and Phenotyping including Anti-D Blend, = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification= no overall result interpretation, only well result shown as DP For Crossmatching = Incompatible	Agglutinated RBCs as a line at the top of the gel or dispersed in upper part of the gel and non-agglutinated RBCs forming a pellet at the bottom of the well. The instrument interpretation software displays “DP” (double population) for a mixed field result.
?	For Blood Grouping including Reverse ABO Testing, Antisera, and Phenotyping including Anti-D Blend, Antibody Detection and Identification, Direct Antiglobulin Testing = Not interpretable For Crossmatching = Incompatible	Ambiguous result.

* RBCs = Red Blood Cells

EXPECTED REACTIONS:

IH-Anti-D (RH1)Blend Result Interpretation	RhD Interpretation
Positive	D antigen present
Negative	D antigen not present

QUALITY CONTROL

On each day of use, the reactivity of all Blood Grouping Reagents should be confirmed by testing with known positive and negative samples. The Blood Grouping Reagent contained on this card could be controlled by testing Rh(+) and Rh(-) samples. Weak D samples may also be used for the positive control when available. Each reagent is satisfactory for use if positive and negative samples react as expected. For additional information, please refer to **IH**-COM User Manual NA and **IH-**1000 User Manual NA, Quality Control sections.

LIMITATIONS

Erroneous and abnormal results may be caused by:

- Bacterial or chemical contamination of the blood specimens, reagents, supplementary materials and/or equipment.
- Patient medication or disease yielding a cross-reaction.
- A red blood cell concentration or suspension medium different from that recommended.
- Incomplete resuspension of the red blood cells.
- Sample hemolysis prior to testing.
- Contamination between microtubes through pipetting errors.
- Use of procedure other than the one described above.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Fibrin, clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the gel and may cause an anomalous result.
- Red blood cells with positive Direct Anti-Human Globulin Test with Anti-IgG reagents may produce false positive results with the IH-Card AHG Anti-IgG.

Please refer to the **IH**-1000 User Manual NA for instrument specific assay limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

The final release testing is performed according to the product specific Standard Operating Procedures. As part of the lot release process, each lot of Bio-Rad Blood Grouping Reagents is tested against antigen positive and negative samples to ensure suitable reactivity and specificity. The IH-Anti-D (RH1) Blend is tested against a panel of D variant samples including weak D types 1-4 and DVI as part of the routine lot release process. Test results with additional D variant samples can be furnished upon request.

Performance characteristics on the IH-1000 Analyzer

Testing to determine the performance characteristics of the Bio-Rad IH-Anti-D (RH1) Blend was performed at four different US clinical sites and included patient, cord blood and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagent IH-Anti-D (RH1) Blend in comparison to the FDA licensed reference reagents.

Results of the positive percent agreement and negative percent agreement, with the one sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below. Note: See the **IH**-1000 User Manual NA and **IH**-Com User Manual NA for more information on verification of results.

Test	Results from Clinical Trials			
	Negative Agreement		Positive Agreement	
	N	Point Estimate (one-sided Exact 95% LCL)	N	Point Estimate (one-sided Exact 95% LCL)
IH-Anti D (RH1)Blend	628	99.68% (99.00%)	2,880	99.97% (99.84%)

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at two external sites and one internal site by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 1 operator x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the **IH**-1000 Analyzer. Reproducibility was demonstrated for the IH-Anti-D (RH1) Blend Blood Grouping Reagent within runs, between runs and between sites.

A precision study was conducted internally using three reagent lots x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the **IH**-1000 Analyzer. Precision was demonstrated with all three lots of IH-Anti-D (RH1) Blend Blood Grouping Reagent.

For technical support or further product information, contact Bio-Rad Laboratories, Inc. at 800-224-6723.

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
	Batch code		*In vitro* diagnostic medical device
	Caution, consult accompanying documents		Consult instructions for use
	Manufacturer		use by (YYYY-MM-DD)
	Contains sufficient quantity for <n> test.		Catalog number
	Temperature limitation	VOL	Volume

BIBLIOGRAPHY

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3. John D. Roback, MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.
4. Standards for Blood Banks and Transfusion Services. 27TH Edition. AABB, 2011.

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