

Blood Grouping Reagent

IH-Anti-D (RH1) Blend

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

Product Identification: 77010

IH-Anti-D (RH1) Blend:

[VOL] 1 x 5 mL vial

[REF] 813804100

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 conventional RhD protein. Weak D types are defined by reduced amounts of 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. Red cells of individuals with partial D types are lacking one or more epitopes of the D antigen. This means that individuals possessing the DVI epitope may produce an anti-D to the missing epitope 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) is distributed into the microtubes containing the appropriate reagent(s). After centrifugation 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.

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

REAGENT

[IVD]

OBSERVABLE INDICATIONS

Do not use if markedly turbid.

Do not use damaged vials.

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 use damaged vials
- Do not use if markedly turbid
- 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, discoloration, bubbles, crystals or other artifacts.
- Do not use cards with damaged foil strips.
- Use reagents as furnished.
- Do not use gel cards if the gel matrix is absent or if the liquid level in the microtube is not at or below the gel matrix. A clear liquid layer should be visible on top of the uniform gel matrix in each microtube.
- Cards with dispersed drops observed at the top of the microtube, due to improper storage or shipping conditions, should be centrifuged with the IH-Centrifuge L or IH-Reader 24 with preset time and speed before use. If drops are still observed on top of the microtube after one centrifugation, it is recommended to not use the card.
- The use of diluents other than IH-LISS for the red blood cell suspension may modify the reaction and lead to incorrect test results.
- The use of volumes and/or red blood cell suspension in concentrations other than those indicated in the method may modify the reaction and lead to incorrect test results, i.e., false positive or false negative results.
- 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.
- Consult [downloads.bio-rad.com](https://www.bio-rad.com/downloads) to download the valid version of this instruction for use.

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.

On automated systems, 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 the expiration date of the unit 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 for up to five (5) days post collection for automated testing.

For manual testing, if testing is delayed, EDTA samples may be stored at 2 to 8°C for up to ten (10) days or donor blood collected in CPD, CP2D and CPDA-1 and donor blood stored in additive solutions AS-1 and AS-3 may be tested up to the expiration date indicated on the label of the unit when stored at 1 to 8°C. Cord blood samples may be stored at 2 to 8°C for up to ten (10) days post collection for manual testing. In case of testing with samples without anticoagulant only manual testing is accepted and if testing is delayed, these samples may be stored at 2 to 8°C for up to ten (10) days.

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 or IH-500 User Manual U.S. for sample requirements.

TEST PROCEDURE FOR MANUAL AND 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 and IH-LISS Solution
- Dispenser pipette capable of delivering 1 mL
- Pipettes: 10 µL, 25 µL, 50 µL and 1 mL
- Disposable pipette tips
- Glass or plastic test tubes
- IH-Incubator L for manual working
- IH-Centrifuge L or IH-Reader 24 to centrifuge the IH-Cards at 85g with pre-set time for manual working
- IH-1000 or IH-500 for full automation

Method

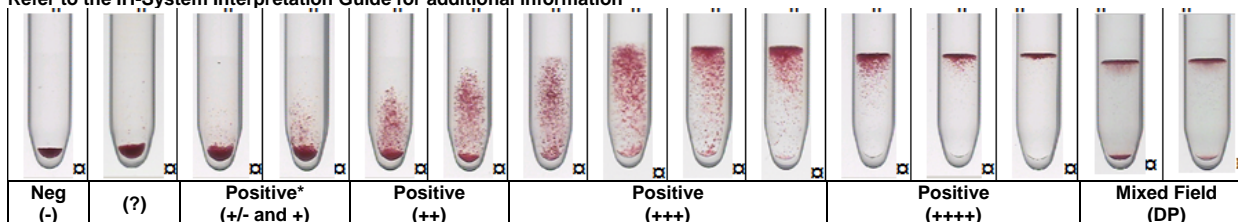
Please refer to the IH-Card AHG Anti-IgG instructions for use for manual testing and the IH-1000 or IH-500 User Manual U.S. for testing and reagent handling instructions.

INTERPRETATION OF RESULTS

For visual interpretation

- Positive result** - Agglutinates on the surface of, or dispersed through the gel. Report as a positive test result if hemolysis is present in the microtube but not in the sample column. Red blood cells may remain suspended on the top of the gel or are dispersed throughout the gel in varying degrees. A few cells may form a button in the microtube bottom in some positive reactions.
- Negative result** - A compact button of red blood cells at the microtube bottom is a negative test result.

Refer to the IH-System Interpretation Guide for additional information



*A very weak reaction is not an expected result for antigen testing. It may indicate that a false positive or a very weak/partial expression of the antigen is present. Further investigation of this sample should be performed before the antigen status is determined.

For automated reading

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, IH-500 User Manual U.S. 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 Antibody Identification = no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible	A pellet of RBCs at the bottom of the well with a very few agglutinated RBCs visible above the pellet or an irregular pellet.
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 at 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

STABILITY OF REACTIONS

For visual reading of reactions, best results are obtained within six (6) hours of centrifugation. Interpretation may be affected by drying of the gel, hemolysis of red blood cells and slanting of reaction patterns due to storage in a non-upright position. Processed cards that are stored in the refrigerator (2 to 8 °C) and properly sealed to protect from evaporation may be interpreted for up to one (1) day. Gel cards should not be interpreted after the first sign of drying, or if hemolysis is observed. The age and condition of red blood cells, as well as the temperature at which the card is stored, will affect how long cards can be stored. The presence of sodium azide in the gel may cause the red blood cells to become dark in color over time. This darkening does not interfere with the test result.

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 D positive and D negative 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-1000 or IH-500 User Manual U.S. and the IH-Com User Manual U.S., 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.
 - Mixed field reaction due to patients pre-transfusion history.
 - 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.
- Strongly lipemic, icteric, hemolytic or microbiologically contaminated samples may lead to false results.
- 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. They may appear as a pinkish layer. In a negative reaction the false appearance of a mixed field could lead to misinterpretation.
- If red blood cells (pellet at the bottom of the microtube) are too low in concentration they become difficult to visualize, and, in certain cases, a weak positive reaction can fail to be detected.
- 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.
- Very weak antigen expression may not be detected. There is no monoclonal Anti-D reagent which will detect all parts of the D antigen.

Please refer to the IH-Reader 24 User Manual or IH-1000, IH-500 and IH-Com User Manual U.S. 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 using IH-1000

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 U.S. and IH-Com User Manual U.S. for more information on verification of results.

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (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 run, 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.

Performance characteristics using IH-500

Testing to determine the performance characteristics of the Bio-Rad IH-Anti-D (RH1) Blend was performed at three different US clinical sites and included patient 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-500 User Manual U.S. and IH-Com User Manual U.S. for more information on verification of results.

Results from Clinical Trials

Test	Sample type	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
IH-Anti-D (RH1) Blend	Random samples	131	100% (97.74%)	691	100% (99.57%)
IH-Anti-D (RH1) Blend	Known RhD neg	250	100% (98.81%)	1	100% (5.00%)
IH-Anti-D (RH1) Blend	Known weak D	NA	NA	39	100% (92.61%)
IH-Anti-D (RH1) Blend	All samples	381	100% (99.22%)	731	100% (99.59%)

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 three external sites 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. Reproducibility for the IH-Anti-D (RH1) Blend Blood Grouping Reagent using the IH-500 was demonstrated within run, between runs and between sites.

Performance characteristics for manual testing

A multi-center clinical trial, which included testing at five different US clinical sites and an internal site, was conducted to evaluate the performance of IH-Anti-D (RH1) Blend. The clinical trial included testing of patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad 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:

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
IH-Anti-D (RH1) Blend	691	99.86% (99.32%)	793	100% (99.62%)

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 three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 2 operators x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the IH-Anti-D (RH1) Blend Blood Grouping Reagent using the IH-Centrifuge L and IH-Incubator L was demonstrated within run, between runs and between sites.

Performance characteristics using the IH-Reader 24

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagent Anti- D (RH1) Blend was performed at five different US clinical sites and one internal site and included patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

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-Reader 24 User Manual and IH-COM User Manual U.S. for more information on verification of results.

Results from Clinical Trials

Test	Negative Agreement N	Negative Agreement (one-sided Exact 95% LCL)	Positive Agreement N	Positive Agreement (one-sided Exact 95% LCL)
IH-Anti-D (RH1) Blend	469	99.79% (98.99%)	795	100% (99.62%)

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 three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 2 operators x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the Blood Grouping Reagent Anti- D (RH1) Blend using the IH-Reader 24 was demonstrated within run, between runs and between sites.

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

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	<i>In vitro</i> diagnostic medical device
!	Consult the instructions for use for important cautionary information such as warnings and precautions	!	Consult instructions for use
M	Manufacturer	e	Use by YYYY-MM-DD
s	Contains sufficient quantity for <n> tests	[REF]	Catalog number
t	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.

Key: Underline = Addition of changes ◀ = Deletion of text