Blood Grouping Reagent

Anti-D (RH1) Blend

Seraclone® Human Monoclonal Blend
(BS232/BS221/H41 11B7)

FOR IN VITRO DIAGNOSTIC USE
For Tube Testing
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1845
Rx only

PACKAGE SIZE

[REF] 802033100 [VOL] 10 x 10 mL Seraclone® Anti-D (RH1) Blend

INTENDED USE

For the determination of the D (RH1) antigen of red blood cells using the tube test. Seraclone® Anti-D (RH1) Blend is suitable for indirect antiglobulin testing.

SUMMARY

The D (RH1) antigen is the most important red blood cell antigen after A and B. Cells that have the D (RH1) antigen are “Rh positive”. Cells that do not have the D (RH1) antigen are “Rh negative”[1]. Soon after the discovery of the Rhesus factor, it became obvious that some red blood cells were weaker reacting with anti-D than other “normal” D-positive red blood cells (Stratton, 1946). These Rhesus antigens were grouped under the heading of D^u. It was also apparent that some D^u red blood cells reacted more strongly with anti-D reagents than others.
The discovery of an allo-anti-D antibody in the serum of a D-positive donor was the first indication that the D antigen may consist - in mosaic fashion - of several different sub-units (epitopes). The Rh(D) antigen of the red blood cells of such persons is described as “partial D”. These -rare- variants have been classified into the categories DII thru DVII, depending on their reactivity with allo-anti-D and monoclonal antibodies.
On the basis of a host of new scientific findings, especially molecular genetic typing the weak expressions of D, originally described as D^u, can now be placed into two groups: partial D like category DII thru DVII or weak D Type 1, 2, 3 etc.
Since 30% to 85% of D negative people who receive a D positive transfusion develop anti-D[2], recipients and donors are routinely tested for this antigen. Some D positive red blood cells require incubation with an anti-D reagent and/or addition of Anti-Human Globulin for agglutination to occur.
The ethnic origin influences the genotype, which can be seen in the table.

Incidence of the More Common Genotypes in D+ Persons[1]

	Genotype	Genotype	Incidence (%)	Incidence (%)
Antigens Present	**DCE**	**Mod. Rh-hr**	**Whites**	**Blacks**
D,C,c,e	**DCe/ce**	**R^1r**	**31.1**	**8.8**
D,C,c,e	**DCe/Dce**	**R^1R^0**	**3.4**	**15.0**
D,C,c,e	**Dce/Ce**	**R^0r'**	**0.2**	**1.8**
D,C,e	**DCe/DCe**	**R^1R^1**	**17.6**	**2.9**
D,C,e	**DCe/Ce**	**R^1r'**	**1.7**	**0.7**
D,c,E,e	**DcE/ce**	**R^2r**	**10.4**	**5.7**
D,c,E,e	**DcE/Dce**	**R^2R^0**	**1.1**	**9.7**
D,c,E	**DcE/DcE**	**R^2R^2**	**2.0**	**1.3**
D,c,E	**DcE/cE**	**R^2r''**	**0.3**	**<0.1**
D,C,c,E,e	**DCe/DcE**	**R^1R^2**	**11.8**	**3.7**
D,C,c,E,e	**DCe/cE**	**R^1r''**	**0.8**	**<0.1**
D,C,c,E,e	**DcE/Ce**	**R^2r'**	**0.6**	**0.4**
D,c,e	**Dce/ce**	**R^0r**	**3.0**	**22.9**
D,c,e	**Dce/Dce**	**R^0R^0**	**0.2**	**19.4**

Bio-Rad Seraclone® Anti-D Blend Blood Grouping Reagent is used to test for the presence or absence of the D antigen in tube test including the Indirect Antiglobulin Test (IAT). Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Bio-Rad Seraclone® Anti-C (RH2), Anti-¢ (RH4), Anti-E (RH3) and Anti-e (RH5) are used principally in the resolution of antibody problems or in family studies.

PRINCIPLES OF THE TEST

The test principle is hemagglutination. The antibodies in Seraclone® Anti-D (RH1) Blend bind to the D antigen on the red blood cells being tested and cause an antigen-antibody reaction visible as red blood cell agglutination.

REAGENT

[IVD]
OBSERVABLE INDICATIONS.
Do not use if markedly turbid.
Do not use damaged vials.

As reactive components Seraclone® Anti-D (RH1) Blend contains human monoclonal antibodies of the immunoglobulin classes IgM and IgG and is therefore suited for an indirect antiglobulin test. The antibodies are derived from cell culture supernatant and demonstrate the consistent specificity and reproducibility characteristic for monoclonal antibodies.
Antibodies are diluted in a buffered protein solution containing bovine albumine.

Seraclone® Anti-D Blend (RH1)
clones BS232/BS221/H41 11B7 (IgM/IgG/IgG)

Preservative: 0.1% sodium azide.

PRECAUTIONS

- For In-vitro diagnostic use.
- Store at 2 to 8°C.
- Do not use beyond the expiration date.
- Do not use if turbid.
- Handle and dispose of reagents as potentially infectious.
- Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
- Caution: This Product Contains Natural Rubber Latex Which May Cause Allergic Reactions.
- Warning: Contains sodium azide (NaN_3), 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.
- The bovine albumin used for the production of this reagent is sourced from donor animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service inspectors to be disease free.
- Consult **downloads.bio-rad.com** to download the valid version of the instruction for use.

Specimen Collection

Fresh samples of clotted, EDTA or citrate anticoagulated whole blood collected following general blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C.
Note: Blood specimens exhibiting gross hemolysis or contamination should not be used.
Clotted samples or those collected in EDTA may be tested within ten days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiration date of the donor unit.

Materials

Materials provided

- Seraclone® Anti-D (RH1) Blend

Materials required but not provided

- Pipettes
- Isotonic saline
- Anti-Human Globulin Anti-IgG (e.g. Bio-Rad Anti-Human Globulin Anti-IgG [REF] 804175100)
- Anti-Human Globulin Anti-IgG, -C3d; Polyspecific (e.g. Bio-Rad Anti-Human Globulin Anti-IgG, -C3d; Polyspecific [REF] 804115100)
- IgG coated red blood cells (e.g. Bio-Rad Coombscell-E [REF] 816030100)
- Negative control (e.g. Bio-Rad Seraclone® Control ABO+Rh [REF] 805171100)
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological centrifuge
- Interval timer
- Markers
- Agglutination viewer (optional)

TEST PROCEDURE

1a. Immediate Spin – Tube Test (optional)

1. Prepare a 3 to 5% suspension of red blood cells to be tested in isotonic saline.
2. Place 1 drop reagent into an appropriately labelled tube.
3. Add one drop (approx 40 to 50 µL) of red blood cell suspension into the tube and mix.
4. Centrifuge for:
   a. 20 seconds at 800 to 1000 x g or
   b. at a time and speed appropriate to the centrifuge calibration.
5. Gently dislodge red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however microscopic examination is not recommended.
6. Record results.

A negative reaction obtained in step 5 must be taken to step 4 in the Tube test method described below.

1b. Tube test – Incubation

1. Prepare a 3 to 5% suspension of red blood cells to be tested in isotonic saline.
2. Place 1 drop reagent into an appropriately labelled tube.
3. Add one drop (approx 40 to 50 µL) of red blood cell suspension into the tube and mix.
4. Incubate 5 to 10 minutes at room temperature (18 to 25°C).
5. Centrifuge for:
   a. 20 seconds at 800 to 1000 x g or
   b. at a time and speed appropriate to the centrifuge calibration.
6. Gently dislodge red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however microscopic examination is not recommended.

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7. Record results.

The negative test obtained in step 6 can be taken to step 4 below.

2. Test for weak D antigen (Indirect Antiglobulin Test)

1. Prepare a 3 to 5% suspension of red blood cells to be tested in isotonic saline.
2. Place 1 drop reagent into an appropriately labelled tube.
3. Add one drop (approx. 40 to 50µL) of red blood cell suspension into the tube.
4. Mix and incubate tube for 15 to 30 minutes at 36 to 38°C.
5. Wash red blood cells 3 times with isotonic saline solution. Completely decant the supernatant.
6. Follow the directions of the Anti-Human Globulin manufacturer.
7. Centrifuge for:
   a. 20 seconds at 800 to 1000 x g or
   b. at a time and speed appropriate for the centrifuge calibration.
8. Gently dislodge the red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however microscopic examination is not recommended.
9. Record results
10. To control all negative antiglobulin tests, add red blood cells sensitized with IgG antibody e.g. Coombscell E (see package insert for procedure).

STABILITY OF REACTION

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

QUALITY CONTROL

The reactivity of all blood typing reagents should be confirmed by testing with known positive and negative red blood cells on each day of use.
To confirm the reactivity or specificity of Bio-Rad Monoclonal Rh Blood Grouping Reagent (Anti-D), it should be tested with antigen-positive (preferably from heterozygous individuals) and antigen-negative red blood cells, respectively. The reagent is satisfactory for use if it reacts only with antigen-positive red blood cells.

A negative control should be performed on samples testing positive with Anti-A, Anti-B and Anti-D. Seraclone® Control ABO+Rh may be used.

INTERPRETATION OF RESULTS

Agglutination of the red blood cells is a positive result and indicates the presence of the corresponding antigen. No agglutination is a negative result and indicates the absence of the corresponding antigen.
An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual)[1].

Result interpretation for RhD Reagent with red blood cells:

Anti-D	Control	D^{weak}Test	DAT**	Interpretation
Positive	Negative	/	/	Rh positive
Negative	Negative	Negative	Negative	Rh negative
Negative	Negative	Positive	Negative	Rh positive*
Negative	Negative	Positive	Positive	Invalid Test
Positive	Positive	/	/	Invalid Test

* A test for weak D may be performed on samples that test negative with Anti-D to determine the Rh status. Certain groups of patients may require testing for weak D. Follow facility specific policies for determining which samples require weak D testing.

**Testing is not valid unless the sample can be shown to react negatively with an appropriate Rh control (e.g. Bio-Rad Seraclone® Control ABO+Rh [REF] 805171100) or exhibits a negative direct antiglobulin test.
Frequencies in the population are listed in the "Summary" section of this package insert.

LIMITATIONS

- Samples with a positive cold agglutinins or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution. False positive results or reaction suspected to be due to cold agglutinins should be resolved according to in-house procedures.
- If the Tube test with Anti-D (RH1) Blend is negative, a test for weak D or partial D may be performed.
- Very weak antigen expression may not be detected. There is no monoclonal Anti-D reagent, which will detect all parts of the D antigen.
- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin.
- 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.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Panagglutinins
  - Improper storage or preparation of red blood cells
  - Mixed field reaction due to patients' pre-transfusion history, real chimeras or chimeras created after bone marrow transplantation
  - Cross reactions with patient's medication (e.g. antibiotics)
  - Contamination of cord blood samples with Wharton's jelly
- Fibrin, clots, particulates or other artifacts may cause an anomalous result.
- Excessive agitation may disrupt weak agglutination and produce false negative results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Testing is performed in accordance with FDA recommended methods.
The final release testing is performed according to the product specific SOPs. As part of the release testing each lot of Bio-Rad Blood Grouping Reagent is tested according to the package insert method against a panel of antigen positive red blood cells (heterozygous antigen expression and weakened antigen expression) to insure suitable reactivity.
The product meets FDA potency requirements.
The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs.
For the product performance it is necessary to adhere to the recommended method in the instructions for use.
The Anti-D reagents have not been tested with rare phenotypes –D-, .D., Rhmod and Rhnull. The performance with enzyme treated red blood cells have not been determined.
If a negative or weak reaction with Bio-Rad Anti-D (RH1) occurs the IAT has to be applied to detect weak D and D category VI antigens. Bio-Rad Anti-D (RH1) Blend is a monoclonal blend of three clones (One IgM and two IgG) suitable for tube technique including Antiglobulin test and detects weak D's and D category VI.
R_0^{HAR} red blood cells were tested and found to be reactive with the monoclonal IgM Anti-D component derived from cell line BS232; but this does not guarantee reactivity of the Seraclone Anti-D Blend Blood Grouping Reagent with all examples of R_0^{HAR} [3,4].
No Blood Grouping Reagent of monoclonal origin has yet been found that will detect all parts of the D antigen.

The performance of the Bio-Rad Anti-D Blend was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

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

NOTE

Manual techniques are to be performed according to the manufacturer's instructions. Each deviation from these instructions is the sole responsibility of the user.
Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	*In vitro* diagnostic medical device
!	Consult the instructions for use for important cautionary information such as warnings and precautions	I	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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2. Frohn C. Dumbgen L, Brand J-M, et al. Probability of anti-D development in D- patients receiving D+ RBCs. Transfusion 2003;43:893-8.
3. The Blood Group Antigen Facts Book. Marion E. Reid and Christine Lomas-Francis. 2nd edition.2008.
4. Hans H. Sonneborn et al. Monoclonal antibodies in research and diagnosis of Rh-system antigens including unusual specificities. Biotest Bulletin 5: 475-483. 1997.

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

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