US

Blood Grouping Reagent

Anti-D (RH1) Blend

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

FOR IN-VITRO DIAGNOSTIC USE
For Tube Testing
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1798

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 Du. It was also apparent that some Du 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) characteristic 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 Du, can now be placed into two groups: partial D like category DII thru DVII or Dweak 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		Incidence (%)	
Antigens Present	**DCE**	**Mod. Rh-hr**	**Whites**	**Blacks**
D,C,c,e	**DCe/ce**	**R_1r**	**31.1**	**8.8**
	DCe/Dce	**R_1R_0**	**3.4**	**15.0**
	Dce/Ce	**R_0r'**	**0.2**	**1.8**
D,C,e	**DCe/DCe**	**R_1R_1**	**17.6**	**2.9**
	DCe/Ce	**R_1r'**	**1.7**	**0.7**
D,c,E,e	**DcE/ce**	**R_2r**	**10.4**	**5.7**
	DcE/Dce	**R_2R_0**	**1.1**	**9.7**
D,c,E	**DcE/DcE**	**R_2R_2**	**2.0**	**1.3**
	DcE/cE	**R_2r''**	**0.3**	**<0.1**
D,C,c,E,e	**DCe/DcE**	**R_1R_2**	**11.8**	**3.7**
	DCe/cE	**R_1r''**	**0.8**	**<0.1**
	DcE/Ce	**R_2r'**	**0.6**	**0.4**
D,c,e	**Dce/ce**	**R_0r**	**3.0**	**22.9**
	Dce/Dce	**R_0R_0**	**0.2**	**19.4**

Biotest Anti-D Blend Blood Group Reagent is used to test for the presence or absence of the D antigen in tube test including the antiglobulin test.
Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Biotest Anti-C (RH2), Anti-c̄ (RH4), Anti-E (RH3) and Anti-e (RH5) are used principally in the resolution of antibody problems or in family studies.

Principle of the Test
The test principle is hemagglutination D. 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
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 purchased from BSE-free US sources, Boval Company L.P. in Cleburne, Tx, USA and Millipore in Kankakee, IL, USA.

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.
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 (drop volume 40 to 50 µl)
- Isotonic saline solution
- Anti-Human Globulin Anti-IgG (e.g. Biotest Anti-Human Globulin Anti-IgG REF 804175100)
- Anti-Human Globulin Anti-IgG,- C3d; Polyspecific (e.g. Biotest Anti-Human Globulin Anti-IgG,- C3d; Polyspecific REF 804115100)
- IgG coated red blood cells (e.g. Biotest Coombscell-E REF 816030100)
- Negative Control (e.g. Biotest Seraclone® Control ABO+Rh REF 805171100)
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological Centrifuge
- Interval Timer
- Markers
- Optical aid (optional). The use of an optical aid for agglutination reading must be vaildated by the user.

Test Procedure
Tube 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 labeled tube.
3. Add one drop of red blood cell suspension into the tube and mix.
4. Centrifuge for 20 seconds at 800 -1000 x g.
5. Gently dislodge red blood cell button and observe for agglutination.
6. The test for weak D antigen should be performed on all donor samples that give a negative or doubtful positive reaction. Proceed to test for weak D.
7. Record results.

The negative test obtained in step 5 can be taken to step 4 below.

Test for weak D antigen
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 labeled tube.
3. Add one drop of red blood cell suspension into the tube.

4. Mix and incubate tube for 15 to 30 minutes at 37°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 20 seconds at 800 -1000 x g.
8. Gently dislodge the red blood cell button and observe for agglutination.
9. Record results

Stability of the 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 Biotest 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.
Negative results in an antiglobulin test should be verified with IgG coated red blood cells: Add 1 drop of IgG coated red blood cells, mix and centrifuge for 20 seconds at 800 -1000 x g. Positive result: The negative reaction in the indirect antiglobulin test is valid, reactive Anti-Human Globulin is present. Negative result: A technical error was made and the test must be repeated.

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 Technial Manual, 15th edition).

Reagent sera with patient red blood cells				
Anti-D	Control	D^{weak} Test	DAT**	Interpretation
+	0	/	/	Rh positive
0	0	0	0	Rh negative
0	0	+	0	* Rh positive
0	0	+	+	Invalid Test
+	+	/	/	Invalid Test

+ = agglutination 0 = no agglutination

* 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. Biotest Seraclone® Control ABO+Rh REF 805171100) or exhibits a negative direct antiglobulin test.
Frequencies in the population are listed in the "Summary" section.

Limitations
- Samples with a positive direct antiglobulin test, 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. It is recommended that an appropriate control be tested in parallel.
- If the immediate reaction with Anti-D (RH1) Blend is negative, a test for weak D antigen (D^{weak} or $D^{variant}$) may be performed.
- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-IgG Solidscreen II.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold Antibodies

Specific Performance Characteristics
Testing is performed in accordance with FDA recommended methods.
The final release testing is performed according to the product specific SOPs. Each lot of Biotest blood group reagent is tested in the Quality control by package insert method against a panel of antigen positive red blood cells (heterozygous antigen expression and if possible weakened antigen expression) to insure suitable reactivity. The products meet 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 reactions with enzyme treated red blood cells has not been determined.
If a negative or weak reaction with Biotest Anti-D (RH1) occurs the IAT has to be applied to detect weak D and D category VI antigens. Biotest Anti-D (RH1) Blend is a monoclonal blend of three clones (One IgM and two IgG) suitable for tube technique including Antiglobulin test and detect weak D's and D Category VI.
No blood grouping reagent of monoclonal origin has yet been found that will detect all parts of the D antigen.

The performance of the Biotest 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 Biotest Diagnostics Corporation at 800-522-0090.

Note
Each facility should verify the optimum spin time for the specific centrifuge in use.
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
⚠	Caution, consult accompanying documents		Consult instructions for use.
	Manufacturer		Use by YYYY-MM-DD
	Contains sufficient quantity for <n> tests.	REF	Catalog number
	Temperature limitation	VOL	Volume

Bibliography

1. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005.
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.

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