Blood Grouping Reagent
Anti-D (RH1)
Seraclone® Human Monoclonal
(BS 226)

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

Package size
REF 802042100 VOL 10 x 10 mL Seraclone® Anti-D (RH1)

Intended Use
For the determination of the D (RH1) antigen of red blood cells using the tube test.

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”. 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'. It was also apparent that some D' 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) antigens 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 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, 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 antihuman 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

<table>
<thead>
<tr>
<th>Antigens Present</th>
<th>Genotype</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D,C,c,e</td>
<td>DCE/ce</td>
<td>31.1</td>
</tr>
<tr>
<td>D,C,e</td>
<td>DCE/DCE</td>
<td>17.6</td>
</tr>
<tr>
<td>D,c,e</td>
<td>DcE/ce</td>
<td>10.4</td>
</tr>
<tr>
<td>D,c,e,e</td>
<td>DcE/DcE</td>
<td>2.0</td>
</tr>
<tr>
<td>D,c,c,e,e</td>
<td>DcE/Dce</td>
<td>11.8</td>
</tr>
<tr>
<td>D,c,e</td>
<td>Dhce/Dhe</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Bio-Rad Seraclone® Anti-D Blood Grouping Reagents are used to test for the presence or absence of the D antigen. Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Bio-Rad Anti-C (RH2), Anti-c (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. The antibodies in Seraclone® Anti-D (RH1) bind to the D antigen on red blood cells and cause an antigen-antibody reaction visible as red blood cell agglutination.

Reagent
As the reactive component Seraclone® Anti-D BS226 contains human monoclonal antibody of the immunoglobulin class IgM and is therefore not suited for an indirect antiglobulin test. The antibody is derived from cell culture supernatant and demonstrates the consistent specificity and reproducibility characteristic for monoclonal antibodies. Antibodies are diluted in a buffered isotonic saline solution containing bovine albumine and macromolecular potentiators. Seraclone® Anti-D (RH1) clone BS226 (IgM)
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 (NaN₃), 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 animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service inspectors to be disease free.

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)

Materials required but not provided
- Pipettes
- Isotonic saline
- Negative control (e.g. Bio-Rad Seraclone® Control ABC+Rh)
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological centrifuge
- Interval timer
- Markers
- Agglutination viewer (optional).

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 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 for 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, macroscopic examination is not recommended.
6. 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

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).

If a negative or weak reaction with Bio-Rad Anti-D (RH1) occurs the IAT may be applied to detect weak D and D category VI antigens. Bio-Rad Anti-D (RH1) Blend (REF) 802033100 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. 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 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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>LOT</td>
<td>Batch Code</td>
</tr>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>⚠️</td>
<td>Caution, consult accompanying documents</td>
</tr>
<tr>
<td>⚠️</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>⚠️</td>
<td>Use by YYYY-MM-DD</td>
</tr>
<tr>
<td>⚤</td>
<td>Contains sufficient quantity for &lt; &gt; tests.</td>
</tr>
<tr>
<td>⚤</td>
<td>Catalog number</td>
</tr>
<tr>
<td>⚤</td>
<td>Temperature limitation</td>
</tr>
<tr>
<td>VOL</td>
<td>Volume</td>
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Bibliography


Key: _ = Underline = Addition of changes  ❯ = Deletion of text