Bio-Rad Seraclone® Anti-D (RH1) clone 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 (NaN₃), which may react with lead or copper pluming 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 this 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 4 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
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 microscopic examination is not recommended.
6. 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 labelled tube.
3. Add one drop (approx. 40 to 50 µL) 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)

**Result interpretation for RhD Reagent with red blood cells:**

<table>
<thead>
<tr>
<th>Anti-D</th>
<th>Control</th>
<th>D***Test</th>
<th>DAT**</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>/</td>
<td>/</td>
<td>R̄h positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Rh negative</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Rh positive*</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Invalid Test</td>
</tr>
</tbody>
</table>

* 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.**

**STABILITY OF REACTION**

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.

**Glossary of Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>[LOT]</td>
<td>Batch Code</td>
</tr>
<tr>
<td>[IVD]</td>
<td>In vitro diagnostic device</td>
</tr>
<tr>
<td>[REF]</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>[VOL]</td>
<td>Catalog number</td>
</tr>
<tr>
<td>[M]</td>
<td>Manufacture</td>
</tr>
<tr>
<td>[s]</td>
<td>Contains sufficient quantity for &lt;n&gt; tests</td>
</tr>
<tr>
<td>[VOL]</td>
<td>Volume</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**


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