Blood Grouping Reagents

Anti-A (ABO1)
Seraclone® Murine Monoclonal (A003)

Anti-B (ABO2)
Seraclone® Murine Monoclonal (B005)

Anti-A,B (ABO3)
Seraclone® Murine Monoclonal Blend (BS63/BS85)

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

Package size
REF 801325100 VOL 10 x 10 mL Seraclone® Anti-A (ABO1)
REF 801350100 VOL 10 x 10 mL Seraclone® Anti-B (ABO2)
REF 801375100 VOL 10 x 10 mL Seraclone® Anti-A,B (ABO3)

Intended Use
For the determination of the A (ABO1), B (ABO2), A,B (ABO3) antigens of red blood cells using the tube test.

Summary
Between 1900 and 1902, Landsteiner and associates discovered the ABO system of red blood cell antigens. The importance of this discovery is the recognition that antibodies are present when the corresponding antigens are lacking. The ABO system is the only blood group system in which the demonstration of consistent specificity and reproducibility characteristic for monoclonal antibodies. Both antibodies derived from a single clone (sister cells of one hybridoma cell) and a mixture of different antibodies derived from several clones are called monoclonal. Antibodies are diluted in a buffered protein solution containing bovine albumin, ethylenediamine tetraacetate (EDTA), and as colorant Patent Blue (Anti-A) or Tartrazine (Anti-B).

Seraclone® Anti-A (ABO1) clone A003 (IgM)
Seraclone® Anti-B (ABO2) clone B005 (IgM)
Seraclone® Anti-A,B (ABO3) clones BS63/BS85 (IgM/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.
• Handle and dispose of reagents as potentially infectious.
• Caution: Do not pipette by mouth. The absence of murine viruses has not been determined.
• Caution: This Product Contains Natural Rubber Latex Which May Cause Allergic Reactions.
• Warning: Contains sodium azide (Na₃N₃), 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.

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 when tested with Seraclone Anti-A or Seraclone Anti-B and up to 14 days when tested with Seraclone Anti-A,B. Donor blood stored in citrate anticoagulant may be tested until the expiration date of the donor unit.

Materials
Materials provided
• Seraclone® Anti-A (ABO1), Seraclone® Anti-B (ABO2) and/or Seraclone® Anti-A,B (ABO3)

Materials required but not provided
• Pipettes
• Isotonic saline or Phosphate Buffered Solution (PBS). (PBS only when tested with Seraclone® Anti-A,B) 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 saline or PBS.
2. Place one drop reagent into an appropriately labelled tube.
3. Add one drop (approx. 40 to 50 µL) of red blood cell suspension into the labelled tube and mix.
4. Centrifugation 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.

*PBS is only approved for Seraclone® Anti-A,B

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 in false negative or more often weak positive reactions.

Quality Control
The reactivity of all blood grouping 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 ABO Blood Grouping Reagents (Anti-A, Anti-B, Anti-A,B), each should be tested with antigen-positive (preferably from heterozygous or weak antigen expression) and antigen-negative red blood cells, respectively. Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells. Confirmation of results in forward grouping must be obtained by performing the reverse grouping test. A negative control should be performed on samples testing positive with Anti-A, Anti-B and Anti-D. Seraclone® Control ABO®+R 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. Frequencies in the population are listed in the “Summary” section. An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual).

Reaction patterns, red blood cell antigens and isoagglutinins
The interpretation of results in testing infant blood samples may be difficult due to the fact that infant serum does not necessarily contain the natural occurring ABO antibodies for antigens absent from the red blood cells. In all other cases, any discrepancy between forward and reverse grouping has to be resolved before the ABO blood group is recorded. The reagents do not react with crypt cell antigens (T-, Tn-, Tk activated cells). Anti-B reacts correctly negative with acquired B characteristics.

### Reaction pattern

<table>
<thead>
<tr>
<th>Reagent with red blood cells</th>
<th>Reagent Red Blood Cells with serum/plasma</th>
<th>Blood Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>A</td>
</tr>
<tr>
<td>Anti-B</td>
<td>A&lt;sub&gt;2&lt;/sub&gt;, A&lt;sub&gt;2&lt;/sub&gt;*</td>
<td>A,&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Ab</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>B</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>AB</td>
</tr>
</tbody>
</table>

* + = agglutination 0 = no agglutination

*Testing with A<sub>1</sub> Reagent Red Blood Cells is not required

### Reactions of Anti-A (ABO1), Anti-B (ABO2) and Anti-A,B (ABO3) with ABO variants

<table>
<thead>
<tr>
<th>Cells</th>
<th>Anti-A (ABO1)</th>
<th>Seraclone® Anti-B (ABO2)</th>
<th>Anti-A,B (ABO3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>+++</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td>A&lt;sub&gt;B&lt;/sub&gt;</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>++++</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;*</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A&lt;sub&gt;B&lt;/sub&gt;</td>
<td>++(+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>B weak</td>
<td>0</td>
<td>++(+)</td>
<td>++(+)</td>
</tr>
<tr>
<td>A&lt;sub&gt;B&lt;/sub&gt; weak</td>
<td>+</td>
<td>++</td>
<td>++(+)</td>
</tr>
</tbody>
</table>

In very rare cases, it has been observed that when using Seraclone® Anti-A minor traces of A-blood group substance were detected on B-erythrocytes. If this B(A) phenomenon does occur, it should be solved like other discrepancies between blood group determination and reverse typng.

### 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.
- Incubation for 20 minutes may be performed to enhance weak reactions.
- A or B subgroups with very weak antigen expression may not be detected.
- Seraclone® Anti-B reacts correctly negative with acquired B characteristics.
- In case of ambiguous results it is recommended to wash red blood cells at least 2 times.

### Bibliography

---

*Bio-Rad Medical Diagnostics GmbH*

Industriestr. 1, D-63303 Dreieich, Germany

www.bio-rad.com, techsupport.bmd@bio-rad.com

Tel.: +49-6103-3130-0, Fax: +49-6103-3130-724