BLOOD GROUPING REAGENT
Anti-M
ALBAclone®
(Murine Monoclonal IgG)
For Tube Technique

REF Z171U

- FOR IN VITRO DIAGNOSTIC USE
- Meets FDA potency requirements
- Discard if turbid
- Preservative: 0.1% (w/v) sodium azide

CAUTIONS: THE ABSENCE OF ALL VIRUSES HAS NOT BEEN DETERMINED. THIS PRODUCT HAS COMPONENTS (DROPPER BULBS) CONTAINING DRY NATURAL RUBBER.

INTERPRETATION OF LABELING SYMBOLS

<table>
<thead>
<tr>
<th>LOT</th>
<th>Batch code</th>
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<tbody>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
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<tr>
<td><a href="http://www.quotientbd.com">www.quotientbd.com</a></td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
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INTENDED USE
This Anti-M reagent is for the in vitro detection and identification of the human M blood group antigen by direct agglutination.

SUMMARY AND EXPLANATION
The MN status of red blood cells is defined by the amino acid sequence of the major red cell sialoglycoprotein, glycophorin A. Anti-M and anti-N react with their respective antigens on glycophorin A, causing agglutination of the red blood cells and classifying these cells into three distinct phenotypes: M+N-, M+N+ and M-N+. Additionally, irrespective of the MN status of their major glycoprotein, almost all human red blood cells carry the ‘N’-antigen on a minor red blood cell sialoglycoprotein, glycophorin B.

PRINCIPLE OF THE TEST
When used by the recommended technique, this reagent will cause the agglutination (clumping) of red blood cells carrying the M antigen. Lack of agglutination demonstrates the absence of the M antigen.

REAGENT DESCRIPTION
The main component of this reagent is derived from the in vitro culture of the IgG secreting mouse hybridoma:

<table>
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<tr>
<th>Product Name</th>
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<th>Cell Line</th>
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<tbody>
<tr>
<td>Anti-M</td>
<td>Z171U</td>
<td>LM1</td>
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</table>

The formulation also contains bovine serum albumin, EPPS buffer and 0.1% (w/v) sodium azide.

NOTE: The volume delivered by the reagent bottle dropper is approximately 40 µL. Care should be taken to ensure that appropriate serum to cell ratios are maintained in all test systems.

STORAGE
The reagent should be stored at 2-8 °C.

WARNINGS AND PRECAUTIONS
For in vitro diagnostic use only

- Products should be used by qualified personnel
- Do not use beyond the expiration date
- Do not use if turbid
- Do not dilute

The format of the expiration date is expressed as YYYY-MM-DD (Year-Month-Day)

- This reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide buildup.
- This reagent is of animal origin, therefore care must be taken during use and disposal as there is a potential infection risk.

CAUTION: SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED, WAS FOUND NEGATIVE FOR INFECTIOUS AGENTS WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS.

PROCEDURE

NOTE: This reagent has been standardized for use by the technique described below and therefore its suitability for use by other techniques cannot be guaranteed. When a test is required to be incubated for a specific time period, a timer should be used. It is recommended to allow the reagent to reach 20-25 °C prior to use.

When using supplemental testing equipment (i.e. centrifuge), follow the procedures that are contained in the operator’s manual provided by the device manufacturer.

Tube Technique - 5 Minute Incubation/Spin

All red blood cells to be tested with this reagent should be washed at least once and resuspended in unbuffered isotonic saline. This includes red blood cells used for quality control.

1. Prepare a 2-4% suspension of red blood cells in unbuffered isotonic saline solution, 9 g/L NaCl.
2. Add 1 drop of blood grouping reagent to a glass test tube.
3. Add 1 drop of red blood cell suspension. Steps 2 and 3 may be performed in either order.
4. Mix the contents of the test tube and incubate at 20-25 °C for 5 minutes.
5. Centrifuge the test tube.
   NOTE: Suggested centrifugation: 900-1000 g (approx. 3400 rpm) for 10 seconds or a time and speed appropriate for the centrifuge used that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy re-suspension of antigen-negative red blood cells.

6. After centrifugation, gently shake the tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination. Do not use any optical aid to examine the tests results.

7. Record results.

STABILITY OF REACTION
Test results should be read, interpreted and recorded immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes resulting in weak positive or false negative reactions.

INTERPRETATION OF RESULTS
Agglutination = positive test result
No agglutination = negative test result

QUALITY CONTROL
Quality control of reagents is essential and should be performed on each day of use and in accordance with local, state and federal regulations.

M+N+ red blood cells should be used as a positive control
M-N+ red blood cells should be used as a negative control

False positive test results are rarely seen with low-protein reagents. False positive agglutination may be due to a positive direct antiglobulin test (DAT), cold agglutinins, or abnormal serum proteins. If false positive results are suspected, or local regulations require, and a control test for spontaneous agglutination is desired, ALBAcheck® - BGS Monoclonal Control (Z271U) or 6-10% albumin in saline may be substituted for the blood grouping reagent in the testing procedure. A negative result would serve as an appropriate control. If the monoclonal control test gives a positive reaction, a valid interpretation of the results obtained in red blood cell testing cannot be made without further investigation.

LIMITATIONS
As this reagent reacts optimally at pH 8.5 and is extremely sensitive to pH, test red blood cells should be suspended in unbuffered medium. All red blood cells suspended in buffered medium e.g. Modified Alsever’s solution, should be washed at least once and resuspended in unbuffered saline prior to use.

Incubation at temperatures above that recommended may result in weaker reactions.

Cells modified by proteolytic enzymes must not be used, as M antigens may be destroyed.

Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.

The expression of certain red blood cell antigens may diminish in strength during storage, particularly in EDTA and clotted samples. Better results will be obtained with fresh samples.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

Suppressed or weak expression of blood group antigens may give rise to false negative reactions.

Gently re-suspend tube tests before reading. Excessive agitation may disrupt weak agglutination and produce false negative results.

SPECIFIC PERFORMANCE CHARACTERISTICS
Prior to release, each lot of ALBAclone® Anti-M is tested using FDA recommended methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity.

During comparator studies (data on file at Alba Bioscience Limited), blood samples were tested with ALBAclone® Anti-M (Monoclonal) as follows*:

<table>
<thead>
<tr>
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<th>Comparator Reagent</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
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<tr>
<td>Trial Reagent</td>
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<tr>
<td>Positive</td>
<td>81</td>
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<tr>
<td>Negative</td>
<td>0</td>
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<tr>
<td>Total</td>
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<table>
<thead>
<tr>
<th></th>
<th>Positive Percent Agreement*</th>
<th>100</th>
<th>98.37</th>
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<tbody>
<tr>
<td></td>
<td>Negative Percent Agreement*</td>
<td>100</td>
<td>85.41</td>
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* The data presented in this table was generated during field trials executed in support of the original US licensing of this reagent.

BIBLIOGRAPHY
2. AABB Standards Program Committee: Standards for Blood Banks and Transfusion Services, ed 30. AABB, 2016

DATE OF ISSUE
2020-04-07