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: 0.5% (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

Batch code


Use by (YYYY-MM-DD)

Storage temperature limitation (2–8 °C)

In vitro diagnostic medical device

Consult instructions for use


Manufacturer

Product Code


Warning

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:

Product Name	Product Code	Cell Line
Anti-M	Z171U	LM1

The formulation also contains bovine serum albumin, EPPS buffer and 0.5% (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.

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 0.5% (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.

Harmful if swallowed.
Harmful in contact with skin.
Wear protective gloves/protective clothing.
Refer to Safety Data Sheet for further information.
Dispose of contents in accordance with local, state or national legislation.

This reagent contains material of animal origin (murine and bovine), 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.

The bovine material used in the manufacture of this reagent was collected in a USDA approved facility or obtained from a geographical region classified as having negligible risk for BSE.

Monoclonal antibodies exhibit a high degree of potency, avidity and specificity. When using such antibodies, great care should be taken to avoid cross contamination.

This product has components (dropper bulbs) containing dry natural rubber.

STORAGE

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

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected by a standard collection technique. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at refrigerated temperatures.

Clotted samples, or those collected in EDTA, should be tested within fourteen days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiry date of the donation.

Special care should be taken if hemolyzed samples must be tested. Grossly icteric or contaminated blood specimens should not be used.

MATERIALS

Material provided
- ALBAclone® Anti-M

Materials required but not provided
- Unbuffered Isotonic saline
- Reagent red blood cells suitable for the control of Anti-M
- 10 x 75 mm or 12 x 75 mm glass test tubes
- Pipettes
- Centrifuge
- Timer
- Heating block/waterbath (optional)

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.

Refer to Performance Limitations section for additional guidance on the use of this product

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

Other red blood cell types may be suitable but should be selected with care.

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.

PERFORMANCE 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.

Comparator Study Results

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

Anti-M		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	81	0	81	
	Negative	0	19	19	
	Total	81	19	100	
Positive Percent Agreement*				100	96.37%
Negative Percent Agreement*				100	85.41%

** The data presented in this table was generated during field trials executed in support of the original US licensing of this reagent.*

In a subsequent performance evaluation study conducted at one internal site (data on file at Alba Bioscience Limited), blood samples were tested with ALBAclone® Anti-M as follows:

Reagent	No. Samples Tested	Concordance
ALBAclone® Anti-M	357	100%

A comparator study was conducted to evaluate the performance of the trial reagent against a comparable FDA licensed product. Repeatability and reproducibility of the trial reagent was confirmed by means of Lot to Lot and Precision studies.

Comparator Study Results

During the internal comparator study (data on file at Alba Bioscience Limited), blood samples were tested with ALBAclone® Anti-M (Murine Monoclonal IgG) as follows:

Anti-M		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	177	0	177	
	Negative	0	180	180	
	Total	177	180	357	
Positive Percent Agreement				100	98.32%
Negative Percent Agreement				100	98.35%

In the internal performance evaluation studies, 357 samples were tested with ALBAclone® Anti-M (Murine Monoclonal IgG). The positive percent agreement at the one-sided 95% exact lower confidence limit was 98.32% for agglutination tests based on a comparison of interpreted results. The negative agreement at the one-sided 95% exact lower confidence limit was 98.35% for agglutination tests based on a comparison of interpreted results. The positive and negative percent agreement did not meet the acceptance criteria of 99% at the one sided 95% lower confidence limit due to the low number of samples tested during the study. No discrepancies were noted between the trial and comparator reagents.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

Precision Study Results

As part of the internal performance evaluation, precision and lot to lot studies were performed using multiple operators, days and runs to confirm repeatability and reproducibility of test results in the same run, day and with the same operator and between runs, days and operators. The study took account of variables such as days of the week, times of day and supplementary reagents used in the testing.
All antigen positive test outcomes generated unequivocal positive reactions and antigen negative test outcomes generated unequivocal negative reactions.

BIBLIOGRAPHY

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