BLOOD GROUPING REAGENT

Anti-D *alpha*

ALBAclone®

(Human/Murine Monoclonal IgM)

REF Z031U

For Slide and Tube Techniques

- **Meets FDA potency requirements**
- **Discard if turbid**
- **Preservative: 0.1% sodium azide**

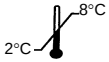
CAUTIONS: THE ABSENCE OF ALL VIRUSES HAS NOT BEEN DETERMINED. THIS PRODUCT HAS COMPONENTS (DROPPER BULBS) CONTAINING DRY NATURAL RUBBER.

INTERPRETATION OF LABELING SYMBOLS

Batch code


Use by (YYYY-MM-DD)

Product code



Storage temperature limitation (2-8 °C)

In vitro diagnostic medical device


Consult instructions for use


Harmful


Manufacturer

SUMMARY

First described in 1939, the RhD antigen is surpassed in importance only by the antigens of the ABO blood group system. Transfusion of RhD positive blood to an RhD negative recipient or failure to administer prophylactic anti-D to an RhD negative woman can result in the production of anti-D. Consequently, establishing the correct RhD group is fundamental to safe transfusion practice. Certain individuals exhibit a quantitative reduction in the expression of their RhD antigen and are categorized as weak D (D^u). Others display a qualitative variation in RhD antigen expression and are referred to as partial RhD. Weak D individuals may also be partial RhD.

This monoclonal IgM Anti-D reagent will directly agglutinate red blood cells from most weak D and partial RhD except DVI.

INTENDED USE

This Anti-D reagent is for the *in vitro* detection and identification of human RhD blood group status by direct agglutination.

PRINCIPLE OF THE TEST

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

REAGENT DESCRIPTION

The main component of this reagent is derived from the *in vitro* culture of the IgM secreting human/mouse heterohybridoma:-

Product Name	Product Code	Cell Line
Anti-D *alpha*	Z031U	LDM1

The formulation also contains potentiators, EDTA and 0.1% (w/v) sodium azide.
The volume delivered by the reagent dropper bottle is approximately 40 µL; bearing this in mind, care should be taken to ensure that appropriate serum: cell ratios are maintained in all test systems.

STORAGE CONDITIONS

The reagent should be stored at 2–8 °C. Do not use if turbid. Do not dilute. The reagent is stable until the expiry date stated on the product label.

PRECAUTIONS FOR USE AND DISPOSAL

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 sink, flush with a large volume of water to prevent azide build-up.
CAUTION: SOURCE MATERIAL FROM WHICH THIS PRODUCT IS DERIVED WAS FOUND NON-REACTIVE FOR HBsAg, ANTI-HIV 1/2 AND ANTI-HCV. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS DISEASE. APPROPRIATE CARE SHOULD BE TAKEN IN THE USE AND DISPOSAL OF THIS PRODUCT.
This product has components (dropper bulbs) containing dry natural rubber. This reagent is for *in vitro* diagnostic use only.

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. Blood specimens exhibiting contamination should not be used. Extreme care should be taken if hemolyzed samples must be tested. Clotted samples or those collected in EDTA should be tested within fourteen days from collection. Donor blood may be tested until the expiry date of the donation.

TEST PROCEDURES

General Information
This reagent has been standardized for use by the techniques described below and therefore its suitability for use in other techniques cannot be guaranteed. When a test is required to be incubated for a specific time period, a timer should be used.

Two tube techniques offering different incubation times are described below. Both are equal and will give comparable results. The user can choose the incubation time within the range that is most compatible with their current laboratory procedures.

ADDITIONAL MATERIALS AND REAGENTS REQUIRED

- Isotonic saline
- Reagent red blood cells for use in RhD grouping
- 10 x 75 mm or 12 x 75 mm glass test tubes or Glass slides
- Pipettes
- Optical aid
- Centrifuge
- Heating block / waterbath
- Timer

RECOMMENDED TECHNIQUES

This reagent and the specimen(s) to be tested should be at room temperature, 20-24 °C, prior to testing.

Tube Technique - Immediate Spin

1. Add 1 drop of blood grouping reagent to a test tube.
2. Add 1 drop of red blood cells suspended to 2-4% in isotonic saline. Reagent red blood cells may be used as provided (preservative suspended).
3. (i) Mix the contents of the test tube well and centrifuge. Suggested centrifugation: 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.
   (ii) After centrifugation, gently shake the tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination.

Tube Technique – 15 minute / spin

1. Add 1 drop of blood grouping reagent to a test tube.
2. Add 1 drop of red blood cells suspended to 2-4% in isotonic saline. Reagent red blood cells may be used as provided (preservative suspended).
3. Mix the contents of the tube test well and incubate for 15 minutes at 37 °C ± 1 °C.
4. (i) Centrifuge the test tube.

Suggested centrifugation: 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.

(ii) After centrifugation, gently shake the tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination.

Slide Technique

1. Add 1 drop of blood grouping reagent to an appropriately prepared area of a glass slide e.g. a wax pencil oval.
2. Add 1 drop of red blood cells suspended to 30-45 % in group homologous plasma/serum.
3. Mix well by rocking the slide for approximately 30 seconds and incubate the test for 5 minutes at 20 – 24 °C with occasional mixing.
4. After incubation, immediately observe macroscopically for agglutination. This may be facilitated by reading over a diffuse light source.
5. If the result is negative or doubtful and a test for weak D is required repeat the test using the Tube Technique.

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 the day of use and in accordance with local, state and federal regulations. We suggest that the following red blood cell samples are used to control the reactions of this reagent. Other red blood cell types may be suitable but should be selected with care.

O R1r red blood cells are recommended as a positive control
O rr red blood cells are recommended as a negative control

PERFORMANCE LIMITATIONS

The quantity of RhD antigen expressed by weak D individuals varies considerably. While this Anti-D reagent will directly agglutinate red blood cells from most weak D individuals, if it is considered important to test for weak D, a reagent specifically prepared for that purpose should be used.

Slide techniques are not recommended for the detection of weak D or partial D samples. If the detection of antigens exhibiting weakened or modified expression is required, negative slide tests should be confirmed by tube testing.

This reagent is potentiated to aid in the detection of weak D and partial D. Very weak agglutination detected at immediate spin ($\leq$ 1+) should be tested using an alternative reagent by the Indirect Antihuman Globulin Test technique prior to the final determination of the RhD type.

Certain tests performed on unwashed samples (e.g. cord), direct antiglobulin test positive samples, or samples stored and tested at below 20 °C, may exhibit false positive reactions due to the potentiators used in the formulation of this reagent. AlbaCheck Reagent Control for Anti-D (Z271U) may be used as a control reagent or alternatively by substituting 6-10% BSA in saline for the blood grouping reagent in the procedure chosen for use. If the control test gives a positive reaction, a valid interpretation of the results obtained in red blood cell testing cannot be made. A control test should always be used if a sample groups as AB RhD positive.

Driblocks and waterbaths promote better heat transfer and are recommended for 37 °C tests, particularly where the incubation period is 30 minutes or less.

Some very weak D and/or partial D samples may not react with monoclonal Anti-D reagents.

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.

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

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

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.

SPECIFIC PERFORMANCE CHARACTERISTICS

Prior to release, each lot of ALBAclone® Anti-D *alpha* is tested by FDA recommended methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity.

This Anti-D reagent will directly agglutinate red blood cells from most known RhD categories except DVI.

This reagent will also directly agglutinate most weak D and unclassified partial D samples.

TECHNICAL NOTE

- It is important to note that monoclonal anti-D reagents vary widely in their ability to detect both partial D and weak D.
- Patients should not be classified as D positive on the basis of a weak reaction with a single anti-D reagent. If clear positive results are not obtained with two monoclonal anti-D reagents it is safer to classify the patient as D negative.
- Patients of category DVI are the most likely to produce anti-D
- Reagents used to test patients for the RhD antigen should not detect category DVI
- Patients with known partial D status should be regarded as D negative
- Reagents used to test donors for the RhD antigen should detect category DVI
- Donors with known partial D status should be regarded as D positive
- If a weak D or partial D is suspected, then further testing/investigation should be performed to determine the D status of the sample.

BIBLIOGRAPHY

1. Technical Manual. 18th ed. Bethesda, MD: AABB, 2014.
2. Standards for Blood Banks and Transfusion Services. 28th ed. Bethesda, MD, 2012.
3. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. BCSH, 2012.

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