

BLOOD GROUPING REAGENT

Anti-D fusion

ALBAclone®

(Human/Murine Monoclonal IgM/IgG)

For Tube Techniques

REF Z043U

- **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



Batch code



Use by (YYYY-MM-DD)



Product code



Storage temperature limitation (2-8 °C)



In vitro diagnostic medical device



Consult instructions for use

www.quotientbd.com



Manufacturer

INTENDED USE

This Anti-D reagent is for the *in vitro* detection and identification of human RhD blood group status in patient and donor samples by direct agglutination and the indirect antiglobulin test. Refer to Technical Note section for further guidance.

SUMMARY AND EXPLANATION

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 pregnant 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. 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 Anti-D reagent will directly agglutinate red blood cells from most weak D and partial RhD except DVI and, therefore, is suitable for RhD grouping of patient samples. This reagent will also detect DVI and weak D by IAT and, therefore, is also suitable for RhD grouping of donor samples. If the detection of weak D, or Rh DVI red blood cells is required the 15-30 minute incubation/spin technique followed by IAT should be used.

PRINCIPLE OF THE TEST

When used by the recommended techniques, 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/IgG secreting human/mouse heterohybridomas:

Product Name	Product Code	Cell Line
Anti-D fusion	Z043U	LDM1, ESD1

The formulation also contains fetal bovine material and 0.1% (w/v) sodium azide.

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 0.1% (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 build-up.

This reagent is of animal origin (murine and bovine), therefore care must be taken during use and disposal as there is a potential infection risk.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

The bovine material used in the manufacture of this reagent was collected in a USDA approved facility. 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 collected in ACD, CPD, CPDA -1, CP2D, CP2D with AS-3, CPD with AS-1, and CPD with AS-5 may be tested until the expiration 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-D fusion

Materials required but not provided

- Isotonic saline
- Reagent red blood cells suitable for the control of Anti-D
- Polyspecific Anti-Human Globulin/Monospecific Anti-Human IgG
- IgG sensitized red blood cells
- 10 x 75 mm or 12 x 75 mm glass test tubes
- Pipettes
- Optical aid (optional)
- Centrifuge
- Timer
- Heating block/waterbath

PROCEDURES

This reagent has been standardized for use by the techniques 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 reagents to reach 18-24 °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.

For routine typing of patient samples, the tube technique with immediate spin, or 15-30 minute incubation/spin, should be used. If the detection of weak D, or Rh DVI red blood cells is required, the 15-30 minute incubation/spin technique followed by IAT should be used.

RECOMMENDED TECHNIQUES

Tube Technique - Immediate Spin

1. Prepare a 2-4% suspension of red blood cells in isotonic saline solution (Reagent Red Blood Cells may be used directly from the vial or according to the manufacturer's instructions).
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 centrifuge.
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.
5. After centrifugation, gently shake the tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination. Negative reactions may be examined with an optical aid.
6. Record results.

NOTE: If detection of weak D or DVI is required proceed as follows. The test tubes read above may be further tested beginning with step 4 of the IAT technique, or step 4 of the 15-30 minute incubation technique.

Tube Technique – 15-30 Minute Incubation/Spin

1. Prepare a 2-4% suspension of red blood cells in isotonic saline solution (Reagent Red Blood Cells may be used

directly from the vial or according to the manufacturer's instructions.)

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 37 °C ± 1 °C for 15-30 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. Negative reactions may be examined with an optical aid.
7. Record results.
8. Proceed to step 8 of the Indirect Antiglobulin Test, if IAT is required.

Tube Technique - Indirect Anti-Human Globulin Test (IAT)

1. Prepare a 2-4% suspension of red blood cells in isotonic saline solution (Reagent Red Blood Cells may be used directly from the vial or according to the manufacturer's instructions).
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 37 °C ± 1 °C for 15-30 minutes.

Optional Steps (5-7)

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.**
7. **Record results.**
8. Wash the test 3-4 times with a large excess of isotonic saline (e.g. 4 mL of saline per 10 (or 12) x 75 mm glass tube).

NOTE: (i) allow adequate spin time to sediment the red blood cells.

(ii) make sure that the residual saline is removed at the end of each wash.

9. Add 2 drops of Anti-Human Globulin (AHG) reagent to each test tube, or as directed by the AHG manufacturers insert.
10. Mix the contents of the test tube and centrifuge.
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.
11. After centrifugation, gently shake the tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination. Negative reactions may be examined with an optical aid.
12. Record results.
13. The validity of all negative tests should be confirmed using IgG sensitized reagent red cells.
 - a) Add 1 drop of IgG sensitized reagent red blood cells to each negative antiglobulin test.
 - b) Mix the contents of the test tube well and centrifuge.
NOTE: Suggested centrifugation: 900-1000g (approx. 3400 rpm) for 10 seconds or a time and speed appropriate for the centrifuge used that produces the strongest reaction of positive tests, yet allows easy re-suspension of negative tests.

- c) After centrifugation, gently shake the tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination.
- d) Any test which does not show a positive reaction should be considered invalid and repeated.

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 the day of use and in accordance with local, state and federal regulations.

RhD(+) red blood cells should be used as a positive control. Suggested phenotype, R,r.
RhD(-) red blood cells should be used as a negative control. Suggested phenotype, rr.

All negative antiglobulin tests should be controlled using IgG sensitized reagent red blood cells. A positive result indicates the presence of active Anti-IgG. A negative result should be considered invalid and repeated if necessary.

PERFORMANCE LIMITATIONS

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

NOTE: Any saline present after the completion of the wash phase may dilute the Anti-Human Globulin reagent beyond its optimal working concentration. Therefore, it is important to ensure that the maximum amount of wash solution is removed after each centrifugation step.

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. A control test should always be used if a sample groups as AB RhD positive. If 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.

Red blood cells that are direct antiglobulin test positive should not be tested using the Indirect Anti-Human Globulin Test.

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

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 re-suspending 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.

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

Care should be taken when testing red blood cells that have been treated with proteolytic enzymes, as these may produce false positive or false negative results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Prior to release, each lot of ALBAclone® Anti-D fusion is tested using 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 weak D and known RhD categories except DVI and therefore if detection of weak D and DVI is required then the 15-30 minute incubation/spin technique followed by IAT should be used.

Comparator Study Results

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

Anti-D fusion		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	2304	0	2304	
	Negative	0	786	786	
	Total	2304	786	3090	
Positive Percent Agreement*				100	0.99
Negative Percent Agreement*				100	0.99

* Indicates agreement between the ALBAclone® Anti-D fusion and comparator reagent.

In performance evaluation studies, 3090 samples were tested with ALBAclone® Anti-D fusion (Human/Murine Monoclonal IgM/IgG). The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. The negative agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results.

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

During in house studies and performance evaluation, examples of partial RhD and weak D were included in the blood samples tested. The performance characteristics are noted below:

RhD Type	Number tested	Immediate Spin/15-30 min Incubation	Indirect Antiglobulin Test
Weak D	27	+/-	+
DIII	2	+	+
DIV	4	+	+
DV	2	+	+
DVI	10	-	+

Precision Study Results

As part of the 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.

TECHNICAL NOTE

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

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DATE OF ISSUE

2022-10



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Z043UPI/05

