BLOOD GROUPING REAGENT Anti-D blend
ALBaclone®
(Human/Murine Monoclonal IgM/IgG Blend)
For Slide and Tube Techniques

**INTENDED USE**
This Anti-D reagent is for the in vitro detection and identification of human RhD blood group status in patient samples by direct antiglobulin, and donor samples by the indirect antiglobulin test.

**SUMMARY AND EXPLANATION**
First described in 1939, the RhD antigen is surmounted 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*). 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.

**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 human/mouse heterohybridomas:

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

**WARRANTS 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 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. APPROPRIATE CARE SHOULD BE TAKEN IN THE USE AND DISPOSAL OF THIS PRODUCT.

The bovine material used in the manufacture of this reagent was collected in a USDA approved facility.

Contains material of murine origin, therefore, handle appropriately, as the absence of murine viruses has not been determined.

**MATERIALS**
Material provided: ALBaclone® Anti-D blend

Materials required but not provided:
- Isotonic saline
- Reagent D blood cells suitable for the control of Anti-D
- Polyspecific Anti-Human Globulin/Monospecific Anti-IgG
- IgG sensitized red blood cells
- 10 x 75 mm or 12 x 75 mm glass test tubes
- Glass slides
- Pipettes
- Optical aid (optional)
- Centrifuge
- Timer
- Heating block/waterbath

**PROCEDURES**
NOTE: 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.

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

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

This reagent and the specimen(s) to be tested should be at 2-8 °C.

**Tube Technique – Immediate Spin**
1. Prepare a 2-4% suspension of red blood cells in isotonic saline solution (Reagent D 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.

**Tube Technique – 15 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 cells suspension. Steps 2 and 3 may be performed in either order.
4. Mix the contents of the test tube and incubate at 37±1 °C for 15 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.

**Indirect Anti-Human Globulin Test**
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±1 °C for 15-30 minutes.

**Optional Steps**
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 test 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 reagent to each tube.
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 blood cells to each negative antiglobulin test.
   b. Mix the contents of the test well 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 positive
tests, yet allows easy re-suspension of negative tests.

e. After centrifugation, gently shake the tube to
dilute the cell button from the bottom and
immediately observe microscopically for
globulins.

d. Any test which does not show a positive
reaction should be considered invalid and
repeated.

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 whole blood or 1 drop of red blood cells
suspended to approximately 30-45% in group
homologous plasma/serum.
3. Mix well by rocking the slide for approximately 30
seconds and incubate the test at 20-24 °C for
5 minutes with occasional mixing.
4. After incubation, immediately observe microscopically
for agglutination. This may be facilitated by reading
over a diffuse light source.
5. Record results
6. If the result is negative or doubtful and a test for weak
D is required repeat the test using the Tube
Technique.

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.

O Rh red blood cells should be used as a positive control
O rr red blood cells should be used as a negative control
Other red blood cell types may be suitable but should be
selected with care.

All negative antiglobulin tests should be controlled using IgG
sensitized reagent red blood cells. A positive result indicates
the presence of active anti-IgG. Tests in which negative
results are obtained with this procedure should be
considered invalid and repeated if necessary.

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

This reagent is potentiated to aid in the detection of weak D
and partial D. Very weak agglutination detected at
immediate spin (<1x) should be incubated and read after
37 °C incubation (at minimum) or tested by the Indirect Anti-
Human Globulin Test technique (preferably) 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 of 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.

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

Any saline present after the completion of the wash phase
can 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.

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
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.

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

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
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Blood Banks and Transfusion Services, ed 30. AABB,
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3. Reid ML, Lomas-Francis C, Olsson ML: The Blood Group

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