QUOTIENT

RhD VARIANT INVESTIGATION KIT

ALBAclone®

(Human/Murine Monoclonal IgG/IgM)

For Tube Techniques

FOR RESEARCH USE ONLY
NOT FOR DIAGNOSTIC PROCEDURES

REF **Z293**

- **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.

CAUTION: THIS PRODUCT OR ANY OF ITS COMPONENTS ARE **NOT** SUITABLE FOR ROUTINE RhD TYPING OF DONOR/PATIENT SAMPLES.

INTERPRETATION OF LABELING SYMBOLS

LOT — Batch code

Use by (YYYY-MM-DD)

REF — Product Code

2°C – 8°C — Storage temperature limitation (2-8 °C)

www.quotientbd.com — Consult instructions for use

Manufacturer

INTENDED USE

This ALBAclone® RhD Variant Investigation Kit is for the *in vitro* investigation of human RhD variants by indirect and direct agglutination. The product is not suitable for routine RhD typing.

SUMMARY AND EXPLANATION

The use of a kit for investigation of RhD variants is highlighted in the current version of the Guidelines for the Blood Transfusion Services in the United Kingdom.
See the reaction profile for details on the differentiation offered by this kit.

PRINCIPLE OF THE TEST

When used by the recommended techniques, each Anti-D sera in the kit will cause agglutination (clumping) of red blood cells expressing the antigen (D epitope(s)) to which each Anti-D is directed. Lack of agglutination means that the antigen (epitope) to which each antibody is directed is not present. The probable RhD variant will be indicated by review of the pattern of reactivity obtained when testing red blood cell sample with all 12 antibodies.

REAGENT DESCRIPTION

The main components of this reagent are derived from the *in vitro* culture of the IgG secreting human/mouse heterohybridomas: LHM76/58, LHM76/59, LHM174/102, LHM50/2B, LHM169/81, ESD1, LHM76/55, LHM77/64, LHM70/45, LHM59/19 and LHM169/80, and the IgM secreting human/mouse heterohybridoma: LDM1.

The formulation also contains 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 expiration 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 a sink, flush with a large volume of water to prevent azide build-up. Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.

In the EU, harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/container in accordance with local/regional/international regulations.

CAUTION: SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED, WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS (INCLUDING 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 AGENTS. APPROPRIATE CARE SHOULD BE TAKEN IN THE USE AND DISPOSAL OF THIS PRODUCT.

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

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

This reagent is for *in vitro* professional 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.

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.

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® RhD Variant Investigation Kit

Materials required but not provided

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

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

RECOMMENDED TECHNIQUES

NIS/LIS Indirect Anti-Human Globulin Test

NOTE: FOR KIT COMPONENTS A-K, THE FOLLOWING TECHNIQUE SHOULD BE USED

1. Prepare a 2-4% suspension of red blood cells in isotonic saline solution or a 1.5-2.0% suspension in low ionic strength saline (Reagent red blood cells may be used directly from the vial or according to the manufacturer's instructions).
2. Add 2 drops of kit component/reagent to a glass test tube.
3. Add 1 drop of a 2-4% or 2 drops of a 1.5-2.0% 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 minutes.
5. 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.
6. Add 2 drops of Anti-Human Globulin reagent to each tube, or follow directions of the Anti-Human Globulin manufacturer.
7. 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.
8. 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.
9. Record results.
10. 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.

NIS/LIS – Direct Agglutination Test

NOTE: FOR KIT COMPONENT L, THE FOLLOWING TECHNIQUE SHOULD BE USED

1. Prepare a 2-4% suspension of red blood cells in isotonic saline solution or a 1.5-2.0% suspension in low ionic strength saline (reagent red blood cells may be used directly from the vial or according to the manufacturer's instructions).
2. Add 2 drops of kit component/reagent to a glass test tube.
3. Add 1 drop of a 2-4% or 2 drops of a 1.5-2.0% 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 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.

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. It is recommended that a positive and negative control be tested with each Anti-D reagent, using R_1r or R_0 cells, and rr cells, if possible.

PERFORMANCE LIMITATIONS

- Weak D samples may react with all kit components to varying degrees due to variation in antigen site density.
- Driblocks and water baths 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 cell antigens may diminish in strength during storage, particularly in EDTA and clotted samples. Better results will be obtained with fresh samples.
- Tube tests should be read by a 'tip and roll' procedure. Excessive agitation may disrupt weak agglutination and produce false negative results.
- It is important to use the recommended g force during centrifugation as 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.
- Direct antiglobulin test positive samples will react by the indirect antiglobulin test irrespective of their RhD status.
- Refer to reaction profile sheet for additional limitations.
- New RhD variant types may be encountered which have not yet been added to the reaction profile of the kit. If you have tested a sample which gives a clear reaction pattern, but the RhD variant is not present in the kit reaction profile, please contact Quotient to discuss your results.

SPECIFIC PERFORMANCE CHARACTERISTICS

The reaction profile provided applies to all current and previous batches of the ALBAclone® RhD Variant Investigation Kit. We may update the reaction profile between lots – check our website for the most up-to-date version of the reaction profile. www.quotientbd.com

BIBLIOGRAPHY

1. Reid, M.E., Lomas-Francis, C. and Olsson, M.L. (2012). The Blood Group Antigen Facts Book. 3rd Edition. Academic Press.

2. Guidelines for the Blood Transfusion Services in the United Kingdom (2013). UK Blood Transfusion Services. 8th Edition. The Stationary Office.

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INSTRUCTIONS FOR USE

Instructions for Use are available on Quotient website at www.quotientbd.com or can be requested from your Local Distributor, by providing the relevant Product Code stated on product labels and Instructions for Use supplied with the product.

ISTRUZIONI PER L'USO

Le istruzioni per l'uso son disponibili sul sito Web di Quotient, www.quotientbd.com, o possono essere richieste al proprio distributore locale fornendo il codice del prodotto indicato sulle etichette del prodotto e nelle istruzioni per l'uso fornite con il prodotto.

HASZNÁLATI ÚTMUTATÓ

A használati útmutatók elérhetők a Quotient honlapján, www.quotientbd.com, illetve igényelhetők a helyi forgalmazótól
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