Anti-Human Globulin Anti-C3d

ALBAclone®
(Murine Monoclonal)
For Tube Technique

DOES NOT CONTAIN ANTIBODIES TO IMMUNOGLOBULINS

REF **Z360U**

- **FOR *IN VITRO* DIAGNOSTIC USE**
- **Meets FDA potency requirements**
- **Discard if turbid**
- **Preservative: 0.1% (w/v) sodium azide**
- **Detects C3b and/or C3d**

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

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)
IVD	*In vitro* diagnostic medical device
www.quotientbd.com	Consult instructions for use
	Manufacturer

INTENDED USE

Anti-Human Globulin, Anti-C3d is intended for use in the direct antiglobulin test to detect the *in vivo* coating of human red blood cells with C3b and/or C3d components.

SUMMARY AND EXPLANATION

The antiglobulin test was first used in blood group serology by Coombs, Mourant and Race in 1945. The serum of animals immunized with human protein was used to detect 'incomplete' antibodies bound to red blood cells. The ability of antiglobulin reagents to detect human complement components bound to red blood cells was reported by Dacie, Crookston and Christensen in 1957.

The direct antiglobulin test will detect complement component C3 bound to red blood cells *in vivo* in serological conditions such as the presence of autoantibodies, antibodies as a result of a transfusion reaction and hemolytic disease of the fetus and newborn.

PRINCIPLE OF THE TEST

The Anti-Human Globulin Anti-C3d will cause the agglutination of red blood cells coated with human C3d and/or C3b complement components. No agglutination will be observed with red blood cells that are not coated with C3b or C3d.

REAGENT DESCRIPTION

The main component of this reagent is a murine monoclonal antibody to C3d (clone number 3G8).
The formulation contains bovine serum albumin, 0.1% (w/v) sodium azide and Tween 80.
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.

STORAGE

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

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only.
Products should be used by qualified personnel.
Do not use beyond 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.
This product has components (dropper bulbs) containing dry natural rubber.

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

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. Do not use blood specimens that exhibit contamination. 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 expiration date of the donation.

For the Direct Antiglobulin Test it is recommended that testing is performed within 48 hours for blood drawn into EDTA. Blood collected into other anticoagulants may be used (ACD, CPD, CPDA-1, CP2D, CP2D-AS3). Clotted specimens should be tested prior to refrigeration to avoid *in vitro* sensitization with complement.

MATERIALS

Material provided
- Anti-Human Globulin Anti-C3d

Materials required but not provided
- Isotonic saline
- Donor or patient red blood cells
- C3 coated red blood cells
- 10 x 75 mm or 12 x 75 mm glass test tubes
- Pipettes
- Centrifuge
- Timer
- Optical aid (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.

Direct Antiglobulin Test
1. Prepare a 2-4% suspension of red blood cells in isotonic saline.
2. Add 1 drop of the 2-4% suspension of red blood cells to a glass test tube.
3. Wash the test 3-4 times with a large excess of isotonic saline. (*e.g.* 4 mL of saline per 12 (or 10) 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.
4. Add 2 drops of Anti-Human Globulin Anti-C3d to each test tube.
   Mix the contents of the test tube well and centrifuge immediately. 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.
5. After centrifugation, gently shake the test 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. The Anti-C3 reactivity can be enhanced by incubation at 21°C ± 3°C for 5 minutes prior to recentrifugation.
7. Record results.
8. To all negative tests: add 1 drop of C3 coated red blood cells and follow manufacturer's instructions. Any test which does not show a positive reaction should be considered invalid and repeated.

STABILITY OF REACTION

Test results should be read and interpreted immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes resulting in weak positive or false negative reactions.

INTERPRETATION OF RESULTS

Agglutination of the test red blood cells indicates a positive test result with detectable C3d or C3b present on the surface of the red blood cells.

No agglutination of the test red blood cells indicates a negative test result with no detectable C3d or C3b present on the surface of the red blood cells.

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.

Anti-C3 reactivity can be confirmed by testing the Anti-Human Globulin reagent with C3 coated red blood cells.

Any reagent red blood cell with a negative direct antiglobulin test may be used as a negative control, if desired.

LIMITATIONS

NOTE: Any saline present after the completion of the wash phase may dilute the Anti-Human Globulin Anti-C3d 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.

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

Comparator Study Results
During comparator studies (data on file at Alba Bioscience Limited), blood samples were tested with ALBAclone® Anti-Human Globulin Anti-C3d (Murine Monoclonal) as follows:

Anti-C3d		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	135	5	140	
	Negative	0	1399	1399	
	Total	135	1404	1539	
Positive Percent Agreement*				100.00	0.98
Negative Percent Agreement*				99.64	0.99

* *Indicates agreement between the ALBAclone® Anti-Human Globulin Anti-C3d and comparator reagents only and does not indicate which reagent gave the correct result(s).*

In performance evaluation studies, 1539 samples were tested with Anti-Human Globulin Anti-C3d (Murine Monoclonal). The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.98 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. The positive percent agreement did not meet the acceptance criteria of 0.99 at the one sided 95% lower confidence limit due to the low frequency of positive samples encountered during the study and not due to discordant results. Five discrepancies between the trial reagent and the comparator reagent associated with the NPA were observed, all were associated with weak reactivity. One could not be resolved as no investigation was performed by the trial site at the time of the discrepancy; four were possible test errors resulting from weak reactions with the trial and the comparator reagents.

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

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 testing.

There were no discordant results; all expected positive test outcomes generated unequivocal positive reactions and expected negative test outcomes generated unequivocal negative reactions.

Prior to release, each lot of Anti-Human Globulin Anti-C3d is tested using FDA recommended methods against IgG and complement coated red blood cells to ensure suitable reactivity.

BIBLIOGRAPHY

1. Coombs RRA, Mourant AE, Race, RR: A new test for the detection of weak and 'incomplete' Rh agglutinins. *Brit J Exp Path*. 1945; 26: 255-266
2. Coombs, RRA., Mourant, AE, Race RR: *In vivo* iso-sensitization of red cells in babies with haemolytic disease. *Lancet* 1946; 247: 264-266
3. Dacie JV, Crookston JH, Christensen WN : Incomplete cold antibodies: role of complement in sensitization to antiglobulin serum by potentially hemolytic antibodies. *Br J Haem*. 1957; 3:77-87
4. Garratty G, Petz LD: The Significance of Red Cell Bound Complement Components in Development of Standards and Quality Assurance for the Anti-Complement Components of Antiglobulin Sera. *Transfusion* 1976; 16: 297-306
5. Moore JA, Chaplin Jr H: Anti-C3d Antiglobulin Reagents. II. Preparation of an Antiglobulin Serum Monospecific for C3d. *Transfusion* 1974; 14: 416-424
6. Howard JE, Winn LC, Gottlieb CE, *et al.*: Clinical significance of the anti-complement component of antiglobulin antisera. *Transfusion* 1982; 22: 269-272
7. Roback JD, Grossman BJ, Harris T, *et al:* AABB Technical Manual, 18th ed. AABB, 2014

DATE OF ISSUE

2023-02

Alba Bioscience Limited
James Hamilton Way
Penicuik
EH26 0BF
UK

U.S. License 1807

Customer Service Tel: 1-888-284-1901
Product Technical Support Tel: 1-888-228-1990
Customer Service Fax: 1-888-694-5208
E-Mail: customer.serviceUS@quotientbd.com
Web: www.quotientbd.com/us

 Z360UPI/04

QUOTIENT