

**MONOCLONAL ANTI-COMPLEMENT REAGENT  
DIRECTIONS FOR USE**

**Anti-C3d monoclonal: For Direct Tube Techniques.**

**SUMMARY**

Without complement, sensitisation and agglutination by an antibody would be incomplete and ineffectual. The complement system proteins make up a highly complex system involving as many as 24 chemically and biologically distinct entities.

**PRINCIPLE**

When used by the recommended techniques, the reagent will cause direct agglutination (clumping) of red cells coated with C3d (and/or C3b). No agglutination usually indicates absence of C3d (and/or C3b, see **Limitations**).

**REAGENT**

Lorne Monoclonal IgM Anti-C3d blood grouping reagent contains mouse monoclonal anti-C3d, Clone BRIC-8. The reagent is supplied at optimal dilution, for use with all the recommended techniques stated below without the need for further dilution or addition. For lot reference number and expiry date see **Vial label**.

**STORAGE**

Reagent vials should be stored at 2 - 8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. This reagent has undergone transportation stability studies at 37°C and -25°C as described in document EN13640:2002.

**SAMPLE COLLECTION AND PREPARATION**

Samples should be drawn aseptically into EDTA to prevent *in vitro* complement binding and tested as soon as possible. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are preferable to clotted ones. If only clotted samples are available, do not refrigerate them before testing. All blood samples should be washed at least twice with PBS or Isotonic saline before being tested.

**PRECAUTIONS**

1. The reagent is intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagent past the expiration date (see **Vial Label**).
4. Do not use the reagent if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagent has been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagent contains <0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. Materials used to produce the products were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
9. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

**DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES**

For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

**CONTROLS AND ADVICE**

1. It is recommended a positive control (C3d and C3b coated cells) and a negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
3. The use of the reagent and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
4. The user must determine the suitability of the reagent for use in other techniques.

**REAGENTS AND MATERIALS REQUIRED**

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive control (C3d and C3b coated) and negative control cells.
- Test tube centrifuge.
- Volumetric pipettes.

**RECOMMENDED TECHNIQUE**

**A. Direct Antiglobulin Technique (DAT)**

1. Wash 1 volume of test red cells (2–3% suspension in PBS or Isotonic saline) 4 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
2. Add 2 volumes of Lorne Anti-C3d to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination

**INTERPRETATION OF TEST RESULTS**

1. **Positive:** Agglutination of test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of complement (C3d and/or C3b) on the test red cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of complement (C3d and/or C3b) on the test red cells.

**STABILITY OF THE REACTIONS**

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those **recommended**.

**LIMITATIONS**

1. Inadequate washing of red cells may result in neutralisation of the reagent.
2. Following completion of the wash phase excess residual saline may dilute the Anti-C3d reagent, reducing its potency.
3. Positive direct antiglobulin results due to complement sensitisation may not reflect *in vivo* complement fixation if test cells are from a previously refrigerated clotted blood specimen.
4. A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Haemolytic Disease of the Newborn or Auto Immune Haemolytic Anaemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
5. False positive or false negative results may also occur due to:  
Contamination of test materials  
Improper storage, cell concentration, incubation time or temperature  
Improper or excessive centrifugation

**SPECIFIC PERFORMANCE CHARACTERISTICS**

1. The reagent has been characterised by the procedure mentioned in the **Recommended Technique**.
2. Prior to release, each lot of Lorne Monoclonal Anti-C3d is tested by the **Recommended Technique** to ensure suitable reactivity.
3. The anti-C3d potency has been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC):
  - Anti-AHG reference standard 96/666
4. Anti-C3d potency is demonstrated in tests employing cells coated with C3.
5. The presence of contaminating heterospecific agglutinins or antibodies to C4d has been excluded in tests employing red cells of all ABO groups and cells coated with C4d.
6. The Quality Control of this reagent was performed using red cells that had been washed with PBS or Isotonic saline prior to use.
7. The reagent complies with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

**DISCLAIMER**

1. The user is responsible for the performance of the reagent by any method other than those mentioned in the **Recommended Technique**.
2. Any deviations from the **Recommended Technique** should be validated prior to use<sup>5</sup>.

**BIBLIOGRAPHY**

1. Wright MS, Issit PD. Anti-complement and the indirect antiglobulin test. *Transfusion* 1979; 19:688-694.
2. Bruce M, Watt AH, Hare W, Blue A, Mitchell R. A serious source of error in antiglobulin testing. *Transfusion* 1986; 26: 177-181.
3. The anti-complement reactivity low ionic methods as published by FDA. *Recommended Methods for Anti-Human Globulin Evaluation* (revision October 1984).
4. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Fourth Edition 2000, Section 3.

5. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. *Transfusion Medicine*, 1995, **5**, 145-150.

**AVAILABLE REAGENT SIZES**








Vial Size	Catalogue Number
2 ml	427002
1000 ml	427000*

\*This size is For Further Manufacturing Use (FFMU) only and is therefore not CE marked.

For the availability of other sizes, please contact:

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**TABLE OF SYMBOLS**

	<b>Batch Number</b>		<i>in-vitro</i> <b>Diagnostic</b>
	<b>Catalogue Reference</b>		<b>Store At</b>
	<b>Expiry Date</b>		<b>Manufacturer</b>
	<b>Read Pack Insert</b>		