

**MONOCLONAL BLOOD GROUPING REAGENTS  
DIRECTIONS FOR USE**

**Monoclonal Anti-C<sup>w</sup>: For Tube, Bio-Rad-ID, Ortho BioVue and Slide Techniques.**

**SUMMARY**

Levine and Stetson discovered the Rh blood group system in 1940. Apart from D the other major Rh antigens are C, E, c and e. The C<sup>w</sup> antigen is one of the more rare antigens, but Anti-C<sup>w</sup> is a fairly commonly encountered antibody. All the Rh antibodies are clinically significant since they may cause both Transfusion Reactions and Haemolytic Disease of the Newborn.

Rh antigen	Caucasians <sup>2</sup>	Blacks <sup>2</sup>	Finns <sup>2</sup>	Latvians <sup>2</sup>
C <sup>w</sup>	2%	1%	4%	9%

**INTENDED USE**

The reagent is a blood grouping reagent intended to be used to qualitatively determine the presence or absence of the C<sup>w</sup> antigen on the red cells of blood donors or patients requiring a blood transfusion when tested in accordance with the recommended techniques stated in this IFU.

**PRINCIPLE**

This reagent contains antibodies to the C<sup>w</sup> antigen on human red cells and will cause agglutination (clumping) of red cells that carry the C<sup>w</sup> antigen, after centrifugation. No agglutination (no clumping) generally indicates the absence of the C<sup>w</sup> antigen (see **Limitations**).

**REAGENTS**

Lorne Monoclonal Anti-C<sup>w</sup> blood grouping reagent is a reagent containing a human monoclonal IgM antibody, diluted in a phosphate buffer containing sodium chloride (0.6 g%), bovine albumin (6 g%) and a preservative. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user. The reagent is supplied at optimal dilution for use with all 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 BS EN ISO 23640:2015.

**SAMPLE COLLECTION AND PREPARATION**

Blood samples can be collected into EDTA, citrate, CPDA anticoagulants or as a clotted sample. The samples should be tested as soon as possible following collection. If a delay in testing should occur, store the samples at 2-8°C. Samples displaying gross haemolysis or microbial contamination should not be used for testing. Blood samples showing evidence of lysis may give unreliable results. It is preferable (but not essential) to wash all blood samples with PBS or Isotonic saline before being tested.

**PRECAUTIONS**

1. The reagents are 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 reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents 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 reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden, but is not supplied sterile. 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 reagents contain <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 reagents 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 (ideally heterozygous) 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. Weak C<sup>w</sup> antigens may be poorly detected by the slide technique. It is recommended that weak C<sup>w</sup> antigens are tested using the tube technique.
3. When typing red cells from a patient who is diagnosed with a disease that causes the red cells to become coated with antibody or other proteins (such as HDN, AIHA), it is important to test the patient's red cells using Lorne's reagent negative control (Monoclonal D Negative Control (catalogue 650010)).
4. In the **Recommended Techniques** one volume is approximately 50µl when using the vial dropper provided.
5. The use of the reagents 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 reagent is in use.
6. The user must determine suitability of the reagents for use in other techniques.

**REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED**

**Tube Technique**

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Centrifuge capable of spinning at 1000 g for 20 seconds.
- PBS solution (pH 6.8-7.2) or Isotonic saline solution (pH 6.5-7.5).
- Positive (ideally heterozygous) and negative control red cells.

**Bio-Rad-ID Micro Typing Technique**

- Bio-Rad ID-Cards (NaCl, Enzyme tests and Cold Agglutinins).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-CellStab or ID-Diluent 2.

**Ortho BioVue Typing Technique**

- Ortho BioVue System Cassettes (Neutral).
- Ortho BioVue System Centrifuge.
- Ortho 0.8% Red Cell Diluent.

**Microtitre plate Technique**

- Validated "U" well microtitre plates.
- Microtitre plate centrifuge.
- Microtitre plate shaker.

**Slide Technique**

- Glass microscope slides or white card tiles.
- Applicator sticks.
- Timer or stopwatch

**All Techniques**

- Volumetric pipettes.

**RECOMMENDED TECHNIQUES**

**A. Tube Technique**

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume of Lorne reagent and 1 volume of red cell suspension.
3. 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.

**B. Ortho BioVue Typing Technique (Neutral cards)**

1. Prepare a 0.8% suspension of red cells in 0.8% Ortho Red Cell Diluent
2. Remove aluminium foil from as many reaction chambers on Neutral cassettes as needed.
3. Place in appropriate reaction chamber: 50µl of red cell suspension and 40µl of Lorne reagent.
4. Centrifuge cassette(s) in an Ortho BioVue Centrifuge.
5. Read macroscopically for agglutination.

**C. Bio-Rad ID Micro Typing Technique**

1. Prepare a 0.8% suspension of red cells in ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes on NaCl, Enzyme tests and Cold Agglutinins ID-Card(s) as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne reagent.
4. Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
5. Read macroscopically for agglutination.

#### D. Microplate Technique, using "U" wells

1. Prepare a 2-3% suspension of red cells in PBS or Isotonic saline.
2. Place in the appropriate well: 1 volume Lorne reagent and 1 volume of red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Re-suspend the cell buttons using carefully controlled agitation on a microplate shaker
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

#### D. Slide Technique

1. Prepare a 35-45% suspension of red cells in serum, plasma or PBS or Isotonic saline. If this is not possible, whole anti-coagulated blood may also be used as the sample.
2. Place on a labelled glass slide or card tile: 1 volume of Lorne reagent and 1 volume of red cell suspension.
3. Using a clean applicator stick, mix the reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for a 1 minute period.
5. Read macroscopically after 1 minute over a diffuse light, do not mistake fibrin strands as agglutination
6. Any weak reactions should be repeated by the tube technique.

#### INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the Cw antigen on the red cells.
2. **Negative:** No agglutination of the red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of the Cw antigen on the red cells.
3. **Control:** Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitised cells.

#### STABILITY OF THE REACTIONS

1. Tube tests must be read immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
2. Slide tests should be interpreted within one minute to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of reagent.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

#### LIMITATIONS

1. Many monoclonal human IgM Rhesus antibodies have been shown to possess Anti-I/i cold agglutinin activity, particularly with Cord cells or enzyme treated cells. This may become apparent if tests are incubated below the recommended temperature.
2. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions and so caution should always be exercised when assigning genotypes on the basis of test results.
3. 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
  - Deviation from the recommended techniques

#### SPECIFIC PERFORMANCE CHARACTERISTICS

1. Prior to release, each lot of this reagent was tested using the recommended test methods listed in this IFU. The tests complied with the test requirements as stated in the current version/issue of the "Guidelines for the Blood Transfusion Services in the United Kingdom".
2. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
3. The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a UK blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.

#### DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use<sup>4</sup>.

#### BIBLIOGRAPHY

1. Issitt PD. Applied Blood Group Serology, 3<sup>rd</sup> Edition, Montgomery Scientific, Miami, 1985, Chapter 10.
2. Marion E.Reid & Christine Lomas-Francis, Blood Group Antigens & Antibodies, SBB Books, New York 2007; Page 31.
3. AABB Technical Manual, 16<sup>th</sup> edition, AABB 2008.
4. Guidelines for the Blood Transfusion Service in the United Kingdom, 6<sup>th</sup> Edition 2002. The Stationary Office.
5. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of

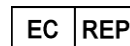
#### AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number	Tests per vial
Anti-C <sup>w</sup> Monoclonal	2 ml	750002	40
	1000 ml	750000*	20,000

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



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