LORNE LABORATORIES LTD.

GREAT BRITAIN

MONOCLONAL BLOOD GROUPING REAGENTS

DIRECTIONS FOR USE

Anti-P_1 Monoclonal: For Tube, Bio-Rad-ID and Ortho BioVue Techniques.

SUMMARY

Landsteiner discovered the P_1 antigen in 1927. Anti-P_1 does not generally react above room temperature and may often go undetected in routine testing. Anti-P_1 does not cause Haemolytic Disease of the Newborn and has only rarely been associated with Haemolytic Transfusion Reactions.

Anti-P_1	Phenotype	Caucasians[2]	Afro-Americans[2]
+	**P_1**	79%	94%
0	**P_2**	21%	6%

INTENDED PURPOSE

The reagent is a blood grouping reagent intended to be used to qualitatively determine the presence or absence of P_1 antigens 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

The reagent contains antibodies to the P_1 antigen on human red cells and will cause direct agglutination (clumping) of red cells that carry the P_1 antigen. No agglutination (no clumping) generally indicates the absence of the P_1 antigen (see **Limitations**).

REAGENT

Lorne Monoclonal IgM Anti-P_1 blood grouping reagent contains mouse monoclonal IgM antibodies prepared from the cell line, Clone 650, diluted in a solution containing sodium chloride and bovine albumin. The reagent does 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 - 8ºC 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 37ºC and –25ºC 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 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, 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 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. 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 P_1 weak cells) and a negative control be tested in parallel with the patient's tests. Results should be considered invalid if controls do not show expected results.
2. In the **Recommended Techniques** one Vial Size 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 reagent is in use.
4. The user must the determine suitability of the reagent for use in other techniques.

REAGENTS AND MATERIALS REQUIRED BUT NOT SUPPLIED

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 P_1 weak) 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.

All Techniques

- Volumetric pipettes.
- Refrigerator set at 2-8ºC.

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. Mix thoroughly and incubate at 2-8ºC for 15 minutes.
4. Centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination

B. 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 a NaCl, Enzyme Tests and Cold Agglutinins gel card as needed.
3. Place in appropriate microtube: 50µl of red cell suspension and 25µl of Lorne reagent.
4. Incubate the ID-Card for 15 minutes at 2-8ºC.
5. Centrifuge ID-Card in a Bio-Rad ID centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique

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 as needed.
3. Place in appropriate reaction chamber: 50µl of red cell suspension and 40µl of Lorne reagent.
4. Incubate the cassette(s) for 15 minutes at 2-8ºC.
5. Centrifuge cassette(s) in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

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 P_1 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 P_1 antigen on the red cells.

STABILITY OF THE REACTIONS

1. Tests should be read immediately after centrifugation. Delays may result in dissociation of antigen-antibody complexes leading to false negative, or weak positive reactions.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. The P_1 antigen is poorly expressed on the cells of newborns.
2. There is a wide variation in the amount of P_1 antigen present on different P_1 positive cells. The strength of agglutination observed with such cells is likely to vary accordingly.

3. Stored blood may give weaker reactions than fresh blood.
4. 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 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 antibody is demonstrated using a panel of antigen-negative cells.
3. The Quality Control of the reagent 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 reagent by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use[5].

BIBLIOGRAPHY

1. Issitt PD. Applied Blood Group Serology, 3rd Edition, Montgomery Scientific, Miami, 1985, Chapter 9.
2. Marion E.Reid & Christine Lomas-Francis, Blood Group Antigens & Antibodies, SBB Books, New York 2007; Page 191.
3. AABB Technical Manual, 16th edition, AABB 2008.
4. Guidelines for the Blood Transfusion Service in the United Kingdom, 6th Edition 2002. The Stationary Office.
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	Tests per vial
2 ml	315002	40
1000 ml	315000*	20,000

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

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