DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
IZ NOE-Sued, Hondastrasse, Objekt M55, 2351 Wr. Neudorf, Austria
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

Anti-A, Anti-B, Anti-AB, monoclonal

(en) English

REF	Content
B054O5	- 1x 10 mL Anti-A, monoclonal
B08405	- 1x 1000 mL Anti-A, monoclonal
B054O6	- 1x 10 mL Anti-B, monoclonal
B08406	- 1x 1000 mL Anti-B, monoclonal
B054O7	- 1x 10 mL Anti-AB, monoclonal
B08407	- 1x 1000 mL Anti-AB, monoclonal

For professional in vitro diagnostic use only.

INTENDED USE

The AB0 reagents are blood grouping reagents intended to be used to qualitatively determine the presence or absence of the A and or B 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.

DIAGNOSTIC SIGNIFICANCE

In 1900, Landsteiner discovered the serum of some people would agglutinate the red cells of others. Four common phenotypes are now recognised: 0, A, B and AB. Subgroups of A and B have since been identified.

Forward Group			Reverse Group				AB0 Phenotype	Caucasians %[1]
A	B	AB	A_1	A_2	B	0		
+	0	+	0	0	+	0	**A**	43
0	+	+	+	+	0	0	**B**	9
0	0	0	+	+	+	0	**0**	44
+	+	+	0	0	0	0	**AB**	4

TEST PRINCIPLE

The reagents contain antibodies against the appropriate A and/or B antigen on human red cells and will cause direct agglutination (clumping) of red cells that carry the corresponding AB0 antigen. No agglutination generally indicates the absence of the corresponding AB0 antigen on human red cells (see LIMITATIONS).

REAGENT COMPOSITION

DIALAB Monoclonal IgM AB0 blood grouping reagents contain mouse monoclonal antibodies diluted in a phosphate buffer containing sodium chloride, EDTA and bovine albumin. 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. Each 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.

Product	Cell Line/Clone	Colour	Dye Used
Anti-A	9113D10	Blue	Patent Blue
Anti-B	9621A8	Yellow	Tartrazine
Anti-AB	152D12 + 9113D10 + ES15	Colourless	None

MATERIAL REQUIRED BUT NOT PROVIDED

- Applicator sticks
- Automatic plate reader
- Bio-Rad ID-Cards (NaCl, enzyme test and cold agglutinins)
- Bio-Rad ID-Centrifuge
- Bio-Rad ID-CellStab or ID-Diluent 2
- Glass microscope slides or white card tiles
- Glass test tubes (10 x 75 mm or 12 x 75 mm)
- Microplate centrifuge
- Ortho BioVue System Cassettes (Neutral)
- Ortho BioVue System Centrifuge
- Ortho 0.8% Red Cell Diluent
- Plate shaker
- PBS solution (pH 6.8 – 7.2) or Isotonic saline solution (pH 6.5 – 7.5)
- Positive and negative control red cells:
- Anti-A: group A (positive control) and group 0 (negative control)
- Anti-B: group B (positive control) and group 0 (negative control)
- Anti-AB: group A and group B (positive controls) and group 0 (negative control)
- Test tube centrifuge.
- Validated “U” well microplates
- Volumetric pipettes

REAGENT PREPARATION

The reagent is ready to use.
Before use, let the reagent warm up to room temperature. As soon as the reagent has been used, put the reagent back in storage at 2-8 °C.

STORAGE AND STABILITY

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 EN ISO 23640:2015.

WARNINGS AND PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- If a reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagents past the expiration date (see vial label).
- Do not use reagent, if a precipitate is present.
- Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
- The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden, but are 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.
- 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.
- 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.

SPECIMEN COLLECTION AND STORAGE

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.

TEST PROCEDURE

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 DIALAB Anti-AB0 reagent and 1 volume of red cell suspension.
3. Mix thoroughly and incubate at room temperature for 1 minute.
4. Centrifuge all tubes for 10 seconds at 1000 rcf or for a suitable alternative time and force.
5. Gently resuspend red cell button and read macroscopically for agglutination.
6. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
7. Following incubation, repeat steps 4 and 5.

B. Bio-Rad-ID Technique (NaCl, enzyme test and cold agglutinins cards)

1. Prepare a 0.8% suspension of red cells in ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50 µL of red cell suspension and 25 µL of DIALAB Anti-AB0 reagent.
4. Centrifuge ID-Card(s) in a Bio-Rad gel card centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique (Neutral cassettes)

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: 50 µL of red cell suspension and 40 µL of DIALAB Anti-AB0 reagent.
4. Centrifuge cassette(s) in an Ortho BioVue System 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 DIALAB Anti-AB0 reagent and 1 volume 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. Resuspend 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.

E. Slide Technique

1. Prepare a 35-45% suspension of red cells in serum, plasma or PBS or Isotonic saline or use anti-coagulated whole blood (in its own plasma).
2. Place on a labelled glass slide or card tile: 1 volume of DIALAB Anti-AB0 reagent and 1 volume of red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 1-minute period, maintaining slide at room temperature.
5. Read macroscopically after 1 minute over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

NOTE:
- Read all tube and microplate tests straight after centrifugation.
- Slide tests should be interpreted after a maximum of one minute to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
- Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

INTERPRETATION OF RESULTS

Positive: Agglutination of the red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate AB0 antigen on the red cells.
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 appropriate AB0 antigen on the red cells.
Discrepancies: If the results obtained with reverse group do not correlate with forward group, further investigation is required.

QUALITY CONTROL AND CALIBRATION

- It is recommended a positive control 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.
- Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive reactions are caused with IgG coated cells.
- Blood specimens of weak A or B subgroups (e.g. Ax) may give rise to false negative or weak reactions when tested using slide, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
- Individuals older than six months should have their AB0 blood-grouping results confirmed by testing their serum or plasma against known group A_1 and B cells before their AB0 blood group can be confirmed.
- In the TEST PROCEDURE one volume is approximately 50 µL when using the vial dropper provided.
- 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 reagents are in use.
- The user must determine the suitability of the reagents for use in other techniques.

PERFORMANCE CHARACTERISTICS

- Prior to release, each lot of DIALAB AB0 monoclonal 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' and the 'Common Technical Specifications'.
- Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
- DIALAB Anti-B does not react with "Acquired-B" red cells.
- DIALAB Monoclonal AB0 reagents do not detect crypt antigens such as T, Tn or Cad.
- The Quality Control of the reagents was performed using red cells with phenotypes that were verified by a blood transfusion centre and had been washed with PBS or Isotonic saline prior to use.

TRACEABILITY

The potency of the reagents has been tested against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-A reference standard 03/188 And / Or Anti-B reference standard 03/164.

EXPECTED VALUES

Expected values are shown in the table in the chapter DIAGNOSTIC SIGNIFICANCE.

LIMITATIONS

- The user is responsible for the performance of the reagents by any method other than those mentioned in the TEST PROCEDURE.
- Any deviations from the TEST PROCEDURE should be validated prior to use.
- AB0 antigens are not fully developed at birth and so weaker reactions may therefore occur with cord or neonatal specimens.
- When using Monoclonal Anti-AB, blood specimens of weak A or B subgroups (e.g Ax) may give rise to false negative or weak reactions when tested using slides, microtitre plates or gel cards. It is advisable to re-test weak subgroups using the tube technique.
- DIALAB monoclonal Anti-A and monoclonal Anti-B are not validated to detect Ax and A_3 or Bx and B_3 antigens, respectively and we therefore do not claim reactivity of the monoclonal Anti-A or Anti-B reagent against these weak A and B sub-groups.
- Stored blood may give weaker reactions than fresh blood.
- 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
  - Cord samples contaminated with Wharton's jelly

WASTE MANAGEMENT

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

LITERATURE

1. Marion E. Reid and Christine Lomas-Francis, Blood Group Antigens and Antibodies, SBB Books, New York 2007; Page 181.
2. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6.
3. Guidelines for the Blood Transfusion Service in the United Kingdom 6th Edition 2002. The Stationery Office.
4. AABB Technical Manual, 16th Edition, AABB 2008.
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.

USED SYMBOLS

Symbol	Description
Cont.	Content