DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Anti-C, Anti-E, Anti-c, Anti-e, monoclonal

Monoclonal Blood Grouping Reagents
For Tube, Bio-Rad-ID, Ortho BioVue, Microplate and Slide Techniques

REF	Content
B06411	1x 5 mL Anti-C, monoclonal
B06412	1x 5 mL Anti-E, monoclonal
B06413	1x 5 mL Anti-c, monoclonal
B06414	1x 5 mL Anti-e, monoclonal

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	Agglutination
Shelf life	24 months from date of production
Storage	2-8°C

INTENDED USE

The Rh reagents are blood grouping reagents intended to be used to qualitatively determine the presence or absence of Rh 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

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 D antigen is highly immunogenic; the C and e antigens are less immunogenic than E and c. The corresponding antibodies are all clinically significant since they may cause both Transfusion Reactions and Haemolytic Disease of the Newborn.

TEST PRINCIPLE

The reagents contain antibodies to the appropriate Rhesus antigen on human red cells and will cause direct agglutination (clumping) of test red cells that carry the corresponding Rh antigen. No agglutination (no clumping) generally indicates the absence of the corresponding Rh antigen (see **LIMITATIONS**).

REAGENT COMPOSITION

DIALAB Monoclonal IgM Anti-Rh blood grouping reagents are low protein reagents containing human monoclonal antibodies diluted with sodium chloride, bovine albumin and macromolecular potentiators (4.0 g%). The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitization or an allergic reaction by the user. Each reagent is supplied at optimal dilution for use with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

Reagent	Cell Line/Clone
Anti-C	MS-24
Anti-E	MS-258
Anti-c	MS-33
Anti-e	MS-16 + MS-63

MATERIAL 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 and negative control red cells:
  Anti-C, monoclonal: R_1r (positive control) and rr (negative control)
  Anti-E, monoclonal: R_2r (positive control) and rr (negative control)
  Anti-c, monoclonal: R_1r (positive control) and R_1R_1 (negative control)
  Anti-e, monoclonal: R_2r (positive control) and R_2R_2 (negative control)

Bio-Rad ID Micro Typing Technique
- Bio-Rad ID-Cards (NaCl, Enzyme and Cold agglutination)
- 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 microplates
- Microtitre plate centrifuge
- Microtitre plate shaker

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

All Techniques
- Volumetric pipettes.

REAGENT PREPARATION

The reagents are 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. The reagent has undergone transportation studies at 37°C and -25°C as described in document EN ISO 23640:2015.

WARNINGS AND 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 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 regents 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 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

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 washed test red cells in PBS or Isotonic saline.
2. Place in a labelled test tube: 1 volume DIALAB Anti-Rh reagent and 1 volume red cell suspension.
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.
5. Any tubes, which show a negative or questionable result, should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 3 and 4.

B. Bio-Rad-ID Technique (NaCl, Enzyme and Cold agglutinins cards):
1. Prepare a 0.8% suspension of red cells in an ID-CellStab or ID-Diluent 2.
2. Remove aluminium foil from as many microtubes on the NaCl/Enzyme/Cold agglutinins ID-Card as needed.
3. Place in appropriate microtube: 50 µL of red cell suspension and 25 µL of DIALAB Anti-Rh reagent.
4. Centrifuge the ID-Card(s) in a Bio-Rad ID centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue 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 on the Neutral cassette as needed.
3. Place in appropriate reaction chamber: 50 µL of test red cell suspension and 40 µL of DIALAB Anti-Rh reagent.
4. Centrifuge cassette(s) for 5 minutes 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-Rh 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 dependent 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 test 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: 1 volume of DIALAB Anti-Rh 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 1 minute, 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:
1. Red all tube and microplate tests straight after centrifugation.
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 the reagent.
3. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

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INTERPRETATION OF RESULTS

1. **Positive:** Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the appropriate Rh antigen on the 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 the appropriate Rh antigen on the test red cells.
3. 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.

QUALITY CONTROL AND CALIBRATION

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. When typing red cells from patients known or suspected to have auto-antibodies, protein abnormalities or a positive Direct Antiglobulin Test (DAT), it is important that a reagent negative control (Anti-D Negative Control, REF: B09936) is tested in parallel.
3. Weak Rhesus antigens may be poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak Rhesus antigens are tested using the tube test technique.
4. In the **TEST PROCEDURE** one volume is approximately 50 µL when using the vial dropper provided.
5. The use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with 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

1. Prior to release, each lot of DIALAB Rh 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”.
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.

LIMITATIONS

1. The user is responsible for the performance of the reagents by any method other than those mentioned in the **TEST PROCEDURE.**
2. Any deviations from the **TEST PROCEDURE** should be validated prior to use[5].
3. DIALAB Anti-Rh reagents are not suitable for use with enzyme treated cells or for use in indirect antiglobulin techniques.
4. Many monoclonal human IgM anti-Rh 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.
5. Some red cells express variant Rh antigens and may give weaker reactions than seen with randomly selected positive control cells. Anti-C may give weaker reactions with C antigen of R_2R_Z individuals. Similarly, Anti-e may give slightly weaker reactions in absence of C antigen, e.g. R_2r, r”r and rr.
6. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative reactions. For these reasons, caution should always be exercised when assigning genotypes on the basis of test results.
7. 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

WASTE MANAGEMENT

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

LITERATURE

1. Issitt PD. Applied Blood Group Serology, 3rd Edition, Montgomery Scientific, Miami, 1985, Chapter 10.
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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.

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