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Anti-D (IgM/IgG)

MONOCLONAL BLOOD GROUPING REAGENTS

For Tube, DiaMed-ID, Microplate and Slide Techniques

REF	Cont.	
B05408	**1x 10 mL**	**Anti-D (IgM/IgG), monoclonal**
B08408	**1x 1000 mL**	**Anti-D (IgM/IgG), monoclonal**

For in-vitro diagnostic use only!

SUMMARY

The Rh blood group system was discovered in 1940. The D antigen is the most clinically significant non-ABO red blood cell antigen and has been implicated in causing Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Anti-D	Phenotype	Caucasians %	Afro-Americans %
+	Rh D positive	85	72
0	Rh D negative	15	28

Table 1: Frequency of each antigen in population.

PRINCIPLE

The reagents will cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D^{VI} in the antiglobulin phase of testing. No agglutination generally indicates the absence of the D antigen (see **LIMITATIONS**).

REAGENT

Dialab Monoclonal Anti-D (IgM/IgG) blood grouping reagent is a low protein, blended reagent containing a human monoclonal IgM and IgG anti-D, diluted in a phosphate buffer containing sodium chloride (0.9 g%), bovine albumin (3 g%) and macromolecular potentiators. When typing patient samples, this reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D^{VI}) and a high portion of weak D (D^{u}) phenotypes when using the recommended techniques. The reagent is supplied at optimal dilution for use on patient samples with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Label.**

IgM/IgG	Cell Line/Clone
IgM	RUM-1
IgG	MS-26

Table 2: Human IgM/IgG Cell Lines/Clones Used

WEAKENED EXPRESSION OF THE ANTI-D

The collective term D^{u} is widely used to describe red cells which have a weaker expression of the D antigen than normal. The term weak D denotes individuals with a reduced number of complete D antigen sites per red cell. The term partial D denotes individuals with missing D antigen epitopes. D^{VI} is a partial D category which misses most D epitopes. Dialab Anti-D (IgM/IgG) reagent will detect most examples of partial and weak D red cells by direct agglutination, but will not detect D^{VI} cells. This reagent will detect D^{VI} and partial D cells in the IAT phase.

STORAGE

Do not freeze. Reagent vials should be stored at 2-8°C on receipt. Pro-longed storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed, store specimens at 2-8°C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested. Blood samples showing evidence of lysis may give unreliable results.

CONTROLS AND ADVICE

1. It is recommended a positive control (ideally R_1r cells), a negative control (ideally rr cells) and a reagent 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 a patient it is important that a reagent negative control is included since the macromolecular potentiators in the reagent may cause false positive reactions with IgG coated cells, e.g. in cases of AIHA or HDN.
3. Test samples for category D^{VI} determination by the Indirect Antiglobulin and Coombs DiaMed-ID Techniques only.
4. Weak and variant D antigens are poorly detected by gel card, microtitre plate and slide techniques. It is recommended that weak and partial variants are tested using the tube test technique.
5. The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.
6. In the **RECOMMENDED TECHNIQUES** one volume is approximately 50 µl when using the vial dropper provided with the 10ml vial.
7. 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.
8. The user must determine the suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Anti-human globulin reagent or Anti-IgG
- Applicator sticks
- Automatic plate reader
- Coombs cell washer
- DiaMed ID-Cards (LISS/Coombs)
- DiaMed ID-Cards (Neutral)
- DiaMed ID-Centrifuge
- DiaMed ID-Diluent: i.e. ID-CellStab
- DiaMed ID-Incubator equilibrated to 37ºc ± 2ºC
- Glass microscope slides
- Glass test tubes (10 x 75 mm or 12 x 75 mm)
- IgG sensitised red cell
- Microplate centrifuge
- Ortho BioVue System Cassettes (AHG/Coombs)
- Ortho BioVue System Cassettes (Neutral)
- Ortho BioVue System Centrifuge
- Ortho BioVue System Heat Block equilibrated to 37ºC ± 2ºC
- Ortho 0.8% Red Cell Diluent
- Plate shaker
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC
- Positive (ideally R1r) and negative (rr) control red cells
- Test tube centrifuge
- Validated "U" well microplates
- Volumetric pipettes
- Water bath or dry heat incubator equilibrated to 37ºc ± 2ºC

RECOMMENDED TECHNIQUES

A. Tube Technique:
1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Dialab Anti-D (IgM/IgG) reagent and 1 volume of test 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 (which can happen with D^{u} or weak D samples), should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 4 and 5.

B. DiaMed-ID Micro Typing Technique (Neutral Cards):
1. Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50 µl of test red cell suspension and 25 µl of Dialab Anti-D (IgM/IgG) reagent.
4. Centrifuge the ID-Card(s) in a Diamed gel card centrifuge.
5. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique (Neutral cards)
1. Prepare a 0.8% suspension of washed test 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 test red cell suspension and 40µl of Dialab ANTI-D (IgM/IgG) 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 washed test red cells in PBS.
2. Place in the appropriate well: 1 volume Dialab Anti-D (IgM/IgG) reagent and 1 volume test 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 test red cells in serum, plasma or PBS.
2. Place on a labelled glass slide: 1 volume of Dialab Anti-D (IgM/IgG) reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20x40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and 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 test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the D antigen 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 the D 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.

STABILITY OF THE REAGENTS

1. Read all tube and microplate tests straight after centrifugation.
2. Complete washing steps without interruption and centrifuge and read tests immediately after addition of anti-human globulin because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
3. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
4. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Dialab Anit-D (IgM/IgG) is not suitable for use with enzyme treated cells or cells suspended in LISS.
2. Stored blood may give weaker reactions than fresh blood.
3. False positive agglutination may be seen when testing IgG sensitised cells.
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. The reagent has been characterised by all the procedures mentioned in the **RECOMMENDED TECHNIQUES**.
2. Prior to release, each lot of Dialab Monoclonal Anti-D (IgM/IgG) is tested by the **RECOMMENDED TECHNIQUES** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Anti-D group reagents for D grouping of patients should not react with D^{VI} cells using the method(s) recommended for use. “Follow-on” tests of negative results using an antiglobulin procedure are not recommended.
4. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
5. The potency of the reagent has been tested against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC):
   - Anti-D reference 99/836
6. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS prior to use.
7. The reagent complies with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusions Services.

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.

PRECAUTIONS

1. The reagent is intended for in vitro diagnostic use only.
2. If a reagent vessel is cracked or leaking, discard the contents immediately.
3. Do not use the reagent past the expiration date (see 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 vessel 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 regent 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 reagent 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 vessel and its contents.

DISPOSAL OF REAGENTS AND DEALING WITH SPILLAGES

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

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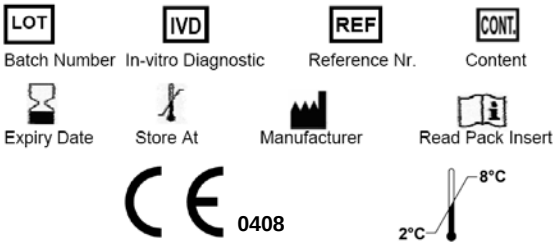
TABLE OF SYMBOLS



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