MONOCLONAL BLOOD GROUPING REAGENTS

CAT NO	DESCRIPTION	PACK SIZE
BGRAA10	MONOCLONAL ANTI A	10x10ml
BGRBB10	MONOCLONAL ANTI B	10x10ml
BGRAB10	MONOCLONAL ANTI AB	10x10ml
BGABD30	MONOCLONAL ANTI A, ANTI B, ANTI D Blend	3x10ml
BGABD40	MONOCLONAL ANTI A, ANTI B, ANTI AB, ANTI D Blend	4x10ml

Intended Use:

Monoclonal Blood grouping reagents from Prestige Diagnostics are intended to be used for the detection of blood grouping antigens in slide, tube and microplate techniques. Anti A cell line BRMA-1 will detect A antigen, Anti B cell line LB-2 will detect B antigen and Anti AB cell lines ES-4/ES-15 will detect A, Ax or B antigens.

Appearance, Preparation and Stability:

Anti A is coloured Blue, Anti B is coloured Yellow and Anti AB is not coloured.
Unopened products are stable up to when stored tightly capped at 2-8ºC. Opened vials are also stable up to expiry when stored without contamination and tightly capped at 2-8ºC.

Specimen Collection:

No Special preparation of the patient is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique. Test the specimen thus collected immediately. Store specimen at 2-8ºC in case the specimen cannot be tested immediately. Specimens exhibiting gross haemolysis, microbial contamination should not be tested with this reagent.

Materials required but not provided:

Microscopic slide, Timer, Isotonic Saline, Test tubes, Centrifuge, Microplate, Microplate shaker, automated readers.

Precautions:

1. The cell lines used to produce these reagents are of murine origin and have been tested and found to be negative for Mouse antibody viruses. Care must be taken in the use and disposal of each container and its contents.
2. These reagents contain 0.1 w/v of Sodium Azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.
3. These products should be clear. Turbidity may indicate bacterial contamination. These reagents should not be used if a precipitate, fibrin gel or particles are present.
4. These reagents are for professional in vitro diagnostic use only.
5. The bovine and porcine materials are obtained from USDA approved sources or from sources which origin information is available. The donor animals are free from and are deemed to have low transmissible spongiform encephalopathy risk.
6. These products should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

Procedure:

Slide Technique:

i) Prepare a 35-50% suspension of test red cells in autologous or compatible plasma, serum or in Isotonic saline.
ii) Add one drop (~40 - 50ul) of either Anti-A or Anti-B or Anti AB reagent to a clean, labelled microscopic slide.
iii) Add one drop (~40 - 50ul) of the suspension of test red cells.
iv) Mix the reagent and the cells over an area of 2cm in diameter by gently and continuously rocking the slide. Read macroscopically after 2 minutes. Do not confuse any dying of the mixture with agglutination.

Tube Technique:

i) Prepare a 3-5% suspension of red cells in isotonic saline.
ii) Add one drop (~40 – 50 µl) of either Anti-A, Anti-B or Anti AB reagent to an appropriately labelled test tube.
iii) Add 1 drop (~40-50 µl) of the suspension of test red cells.
iv) Mix and centrifuge at 1000 rcf for 20 seconds.
v) Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
vi) Incubate weaker than expected reactions for 1 minute at room temperature and then re-spin.

Microplate Technique:

i) Prepare a 3-5% suspension of red cells in isotonic saline.
ii) Add one drop (~40-50 µl) of either Anti-A, Anti-B or Anti AB reagent to an appropriately test wells of a U well Microplate.
iii) Add 1 drop (~40-50 µl) of the suspension of test red cells to the appropriate test wells.
iv) Mix the contents of each well using manual means or a Microplate shaker.
v) Incubate the Microplate at room temperature for 15-20 minutes.
vi) Centrifuge the Microplate at 1000 rcf for 40 seconds.
vii) Resuspend the red cells using the Microplate shaker.
viii) Read tests macroscopically or with an automated reader.

Limitations:

- The results of red cell grouping should be confirmed by reverse grouping the individuals' serum with known A1 and B red cells. No recipient's serum should be given AB blood unless the cells of the recipient are clearly positive with Anti A and Anti B and recipient's serum shown to give negative reactions with A1 and B cells (unless the recipient has been shown to be a subgroup of Ab with Anti A1 in the serum).
- Anti-A blood grouping reagent will not detect all examples of Ax cells. For detection of weaker examples of Ax, microscopic reading and/or a longer incubation of 15 minutes may be required.
- Rigid polystyrene microplates are generally more suitable than those made from PVC. Each batch of microplates should be evaluated in the user's system prior to acceptance as suitable for routine usage.
- False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.

Note:

- It is recommended that a positive control and a negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions.
- It is not required to use a reagent control in parallel with all tests using these reagents. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control recommended. This should be tested in parallel with the reagents.
- These reagents have bee characterised by the procedures recommended in this package insert, their suitability for use in other techniques must be determined by the user.

Performance Characteristics:

Anti-A,B and AB reagents have been tested by each if the recommended techniques with donor, clinical and neonatal specimens collected in EDTA, Citrate, CPDA, SAGM or as a clotted sample. The sample population represented all major ABO phenotypes. The total number of tests (n) and the sensitivity and specificity was calculated for each technique and is shown in the tables below:

Technique	ANTI-A REAGENT			
	n	Sensitivity %	n	Specificity %
TUBE	617	100	708	100
MICROPLATE	4201	100	5844	100
SLIDE	546	99.5	531	100

Technique	ANTI-B REAGENT			
	n	Sensitivity %	n	Specificity %
TUBE	244	100	1083	100
MICROPLATE	1209	100	8838	100
SLIDE	377	100	703	100

Technique	ANTI-AB REAGENT			
	n	Sensitivity %	n	Specificity %
TUBE	748	100	579	100
MICROPLATE	5112	100	4935	100
SLIDE	736	100	343	100

References:

1. Moore S et al., Vox Sang 47: 427-434. A Mouse Monoclonal antibody with Anti-A,(B) specificity which agglutinates Ax cells.
2. McDonald D.F and Thompson, J.M Vox Sang 1991;61:53-58. A New Monoclonal Anti-A Antibody BIRMA-1
3. Issitt, P.D and Anstee D.J. Applied Blood Group Serology, 4th Edition, Montgomery Scientific Publications, 1998.
4. Race R.R and Sanger.R. Blood groups in Man 6th Edition Oxford Blackwell Scientific Publishers 1975.
5. Guidelines for the Blood transfusion services in the United Kingdom, 5th Edition 2001.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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CAT NO	DESCRIPTION	PACK SIZE
BGRDB10	**Anti D Blend**	**10x10ml**

ANTI-D BLEND (IgM/IgG)

Intended Use:

The presence of D antigen is determined by testing the test blood cells against the antibody with a known anti D specificity. The reagent will cause direct agglutination of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D^{VI} in the AHG phase of testing. The agglutination of the red cells being tested indicates the presence in them of the corresponding antigen. No agglutination generally indicates the absence of the D antigen.

Appearance, Preparation and Stability:

This reagent is a blended reagent containing low protein concentrations and a human monoclonal IgM and IgG anti D. This reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D^{VI}) and a high proportion of weak D (D^{u})

Anti D reagent is provided ready to use.

- The reagents will remain stable until the stated expiry date printed on the label, when stored tightly closed at 2-8oC, and contamination is prevented during their use. Do not use the reagents after expiry date.
- Do not freeze or expose to elevated temperatures. Prolonged storage outside the recommended temperature range may result in accelerated loss of reagent reactivity.
- This product should be clear. Turbidity may indicate microbial contamination. Do not use if a precipitate is present.
- If a vial is cracked or leaking, discard the contents of the vial immediately.

Precautions:

Components of different human origin have been tested and found to be negative for the presence of antibodies anti HIV 1+2, Anti HCV as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious. Protective clothing should be worn when handling the reagent, such as disposable gloves. Warning: the reagents in this kit contain sodium azide. Do not allow contact with skin or mucous membranes.

Weakened Expression of the Rh Antigen:

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. The 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 AHG phase.

Specimen Collection:

The blood samples can be collected with or without anticoagulant. They must be tested as soon as possible. Samples collected into EDTA or heparin should be tested within 48 hours. Blood collected into ACD, CPD, CPDA-1 may be typed up to 35 days from the date of withdrawal. Store at 2-8°C.

Materials required but not provided:

Glass test tube, Pasteur Pipettes, Centrifuge, Glass slides, Applicator sticks, PBS: 8.5 – 9 g/l NaCl, pH 7.0 +/- 0.2 at 22+/-1 °C.

Procedure:

Slide Technique:

i) Prepare a 35-50% suspension of test red cells in autologous (or compatible) plasma, serum or in isotonic saline.
ii) Add 1 drop (~40-50ul) of Anti-D reagent to a clean, labelled microscope slide.
iii) Add 1 drop (~40-50ul) of the suspension of test red cells.
iv) Mix the antiserum and cells over an area of about 2 cm in diameter by gently and continuously rocking the slide. Read macroscopically after 3 minutes. Do not confuse drying of the mixture with agglutination.

Negative reaction: No Visible agglutination after 5 minutes

Positive reaction: Positive red cells agglutinate in a few seconds. Do not mistake fibrin strands as agglutination. Any weak reactions should be repeated by the tube technique.

Tube Technique:

i) Prepare a 3-5% suspension of test red cells in PBS.
ii) Place in a glass tube one volume of Anti D Blend and 1 volume of the Red cell suspension and mix well.
iii) Centrifuge at 900 – 1000 rpm for 60 seconds or 3400 rpm for 20 seconds or incubate at room temperature for 20-30 mins.
iv) Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
v) For apparently negative results which are to be tested for D^{VI} and other weak D phenotypes, proceed to step 3 of the indirect antiglobulin test.

Negative reaction: A smooth homogeneous suspension indicates a negative reaction.

Positive reaction: Agglutination of the red cells indicates a positive reaction.

Indirect Anti-globulin technique: AHG test

i) Follow steps i-ii given in the Tube technique.
ii) Mix and incubate at 37°C for 15 minutes.
iii) Wash the cells at least once with PBS, thoroughly decanting the saline.
iv) Add two volumes of AHG or anti IgG to the dry cell button and mix gently to re-suspend the cells. Centrifuge at 900-1000 rcf for 15 seconds.
v) Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
vi) To confirm that negative tests are valid add IgG sensitised red cells (Coombs Control cells), repeat centrifugation and examine for agglutination. If no agglutination is observed the test is invalid and should be repeated.

Positive reaction: Agglutination of the test red cells constitutes a positive test result within accepted limitations, of the test procedure and indicates the presence of D antigen on the test red cells.

Negative reaction: No agglutination of the test red cells constitutes a negative result within accepted limitations of the test procedure and indicates the absence of the D antigen on the test red cells.

Test results of the cells that are agglutinated using the reagent negative control can be excluded, as the agglutination is most probably caused by the effect of the macromolecular potentiators in the reagent on sensitised cells.

Quality Controls:

- It is recommended that a positive control (ideally group R1r cells) and a negative control (ideally group rr cells) be tested in parallel with each batch of tests.
- Tests must be considered invalid if controls do not show expected results.
- 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.
- Read all test tubes straight after centrifugation.
- 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.
- Slide tests should be interpreted within 2 minutes to ensure specificity and to avoid the possibility a negative results 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.
- 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.

Limitations:

- Anti D is not suitable for use with enzyme treated cells or cells suspended in LISS.
- Stored blood may give weaker reactions than fresh blood
- False positive or false negative results may occur due to: Contamination of test materials, Improper incubation time or temperature, improper or excessive centrifugation and improper storage of test materials or omission of reagents.

Performance Characteristics:

- The reagent has been characterised by all the procedures mentioned in this IFU.
- Prior to release, each lot of the reagent is tested by the IFU against a panel of antigen-positive red cells to ensure suitable reactivity.
- The potency of the reagents tested for against the following minimum potency reference standards obtained from NIBSC: 91/592.

References:

1. Kohler G and Milstein C, Continuous culture of fused cells secreting antibody of predefined specificity. Nature. 256: 495-497 (1975)
2. Race RR, Sanger R, Blood groups in Man, 6th Edition, Oxford Blackwell scientific publishers Chapter 2 (1975)
3. Issitt PD. Applied Blood group serology, 3rd Edition, Montgomery scientific, Miami, Chapter 10 (1985)
4. Mollison PL. Blood Transfusion in clinical medicine. 8th Edition, Oxford Blackwell Scientific publications, Chapter7 (1987)
5. Tippett P. Sub-divisions of the Rh (D) antigen. Medical Laboratory Science; 45: 88-93 (1988)

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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