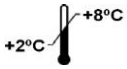
Rare Typing Reagents
Human
Anti-Fy^b



REF IVD

CATALOGUE NUMBER
BG-FYBH2

SUMMARY
The Fy^a and Fy^b antigens were reported in 1950 and 1951 respectively. Anti-Fy^b has been implicated in immediate and delayed Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Anti-Fy^a	Anti-Fy^b	Phenotype	Caucasians %	Afro-Americans %
+	0	Fy(a+b-)	17	9
0	+	Fy(a-b+)	34	22
+	+	Fy(a+b+)	49	1
0	0	Fy(a-b-)	Very Rare	68

PRINCIPLE
The reagent will cause indirect agglutination (clumping) of test red cells, that carry the corresponding Duffy b antigen, in the antiglobulin phase of testing. No agglutination generally indicates the absence of the corresponding Duffy b antigen (see **Limitations**).

REAGENTS
Rapid Labs Human Anti-Fy^b blood grouping reagent is prepared from human serum diluted in a sodium chloride solution containing macromolecular potentiators and bovine albumin. Each 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.

SAMPLE COLLECTION AND PREPARATION
Blood samples be collected into EDTA, citrate, CPDA, anticoagulants or as a clotted sample. The samples should be tested as son as possible following collection. If a delay in testing should occur, store the samples at 2-8ºC. 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 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 reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents 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 reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date.
7. The plasma from which this reagent is manufactured is no longer delipidated, so it is normal for the reagent to have a turbid appearance.
8. 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.
9. Materials used to produce the reagents were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
10. 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.

CONTROLS AND ADVICE
1. It is recommended a positive control (ideally heterozygous cells) 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. The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitised red cells.
3. The reagents contain macromolecular potentiators which may cause false positive reactions with IgG sensitised cells, it is recommended that patient's cells are tested with patient's plasma to test for false positive reactions.
4. Most proteolytic enzymes destroy Fy^b reactivity.
5. In the **Tube Technique** one volume is approximately 50µl when using the vial dropper provided.
6. 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.
7. User must determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED
- Anti-human globulin i.e. Rapid Labs Polyspecific AHG or Rapid Labs Monospecific Anti-IgG
- Coombs cell washer.
- Bio-Rad ID-Cards (LISS/Coombs).
- Bio-Rad ID-Centrifuge.
- Bio-Rad ID-Diluent (ID-CellStab).
- Bio-Rad ID-Incubator equilibrated to 37ºC ± 2ºC.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- IgG sensitised red cells
- Ortho BioVue System Cassettes (AHG Polyspecific or AHG Anti-IgG).
- Ortho BioVue System Centrifuge.
- Ortho BioVue System Heat Block equilibrated to 37ºC ± 2ºC.
- Ortho 0.8% Red Cell Diluent.
- PBS solution (pH 6.8–7.2) or Isotonic saline solution (pH 6.5–7.5).
- Positive (ideally heterozygous) and negative control red cells.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37ºC ± 2ºC.

DIRECTIONS FOR USE

A. Indirect Antiglobulin Technique (IAT)
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 of Rapid Labs reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37ºC for 15 minutes.
4. Wash red cells at least 3 times with PBS or Isotonic saline, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of anti-human globulin or anti-IgG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination
8. Confirm validity of all negative reactions with IgG sensitised red cells.

B. Bio-Rad -ID Micro Typing Technique
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: 50µl of 0.8% test red cell suspension and 25µl of Rapid Labs reagent.
4. Incubate the LISS/Coombs ID-Card(s) for 15 minutes at 37ºC.
5. Centrifuge the LISS/Coombs ID-Card(s) in a Bio-Rad ID centrifuge.
6. Read macroscopically for agglutination.

C. Ortho BioVue Typing Technique
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 on either AHG Polyspecific or AHG Anti-IgG cassettes as needed.
3. Place in appropriate reaction chamber: 50µl of test red cell suspension and 40µl of Rapid Labs reagent.
4. Incubate the cassette(s) for 15 minutes at 37ºC.
5. Centrifuge cassette(s) for 5 minutes in an Ortho BioVue System Centrifuge.
6. Read macroscopically for agglutination.

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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 Duffy 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 appropriate Duffy antigen on the test red cells.

STABILITY OF THE REACTIONS

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results.
2. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those **recommended**.

LIMITATIONS

1. Red cells that have a positive DAT due to a coating of IgG cannot be typed by the **Indirect Antiglobulin Technique.**
2. This reagent contains macromolecular potentiators which may cause false positive reactions with IgG sensitised cells. It is therefore recommended that the patient's cells are tested with the patient's plasma to test for false positive reactions.
3. Antibodies directed at low frequency antigens may occur as unsuspected contaminants in blood grouping antisera. In addition, certain antigens (eg. Bg, Sd^a) can be present in an exalted state on red blood cells. These phenomena may be the source of rare false positive reactions, which may occur with more than one lot of a given specificity.
4. It is not possible to claim the absence of all contaminating antibodies, as red cells carrying antigens of low frequency or exalted antigens are not always available for testing.
5. Suppressed or diminished expression of certain blood group antigens may conversely give rise to false negative results and so caution should always be exercised when assigning genotypes on the basis of test results.
6. False positive agglutination may be seen when testing IgG sensitised cells.
7. False positive results may occur due to Macromolecular potentiators that are present in the reagent
8. 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

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterised by the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Rapid Labs Anti-Fy^b reagent is tested by the **Recommended Techniques** against a panel of antigen-positive red cells to ensure suitable reactivity.
3. The presence of contaminating antibodies to antigens with an incidence of 1% or greater within the random population has been excluded either in tests employing the appropriate antigen-negative red cells or in tests employing the reagents previously absorbed to remove the interfering specificities.
4. Antibodies to Xg^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cell. This can also be said for Yt^b, M^g and V^w and other low frequency antigens which may not be excluded in routine specificity testing and detection will depend upon availability of appropriate test cells
5. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS or Isotonic saline prior to use.
6. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

ANALYTICAL PERFORMANCE

The performance of the reagent has been validated by the test procedures defined in the instructions for use. Any deviations from these techniques are the responsibility of the user

BIBLIOGRAPHY

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4. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6
5. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
6. 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.

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only
REF	Catalogue Number	LOT	Lot Number
+2°C +8°C	Store between 2-8°C		Use by
	Manufacturer		Date of manufacture

Manufactured by: Rapid Labs Ltd

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