BLOOD GROUPING REAGENT

Anti-Fy^b (Monoclonal)

Gamma-clone®

By Tube Test

Preservative: 0.09% Sodium Azide Meets FDA Potency Requirements IVD

CAUTION: THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) MAY CONTAIN DRY NATURAL RUBBER. DO NOT PIPETTE THIS PRODUCT BY MOUTH, AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED.
DO NOT USE IF MARKEDLY TURBID.

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Rx ONLY

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IMMUCOR

Intended Use:

Gamma-clone Anti-Fy^b (Monoclonal) Blood Grouping Reagent is intended for the detection of the Fy^b (FY2) antigen on red blood cells by direct agglutination tube test.

Summary of the Test:

The first example of anti-Fy^a was discovered in 1950 by Cutbush, Mollison and Parkin, in the serum of a patient named Duffy.[1] The antigen recognized by the new antibody was present in approximately 65% of Whites. The antithetical antibody, anti-Fy^b, was first identified in 1951 by Ikin and her fellow-workers.[2] The Duffy system was further expanded in 1956, when Sanger, Race and Jack found that the majority of African-Americans were Fy(a–b–), a phenotype that is exceedingly rare in Whites.[3] In 1965, Chown and associates reported the existence of a variant gene, *Fy^x*, which produces a smaller amount of Fy^b than the *Fy^b* gene.[4] The fact that the product of *Fy^x* may not be recognized by some Anti-Fy^b reagents (and gives weaker-than-normal reactions with others) sometimes causes discrepant results in family studies.
The antibodies of the Duffy blood group system are usually immune in origin, and sometimes bind complement. They usually react only by the indirect antiglobulin technique and have been reported as the cause of hemolytic disease of the newborn and of hemolytic transfusion reactions.

Principle of the Test:

The presence of the Fy^b antigen is determined by testing with Anti-Fy^b by tube test technique. Agglutination of the test red blood cells constitutes a positive test result and indicates the presence of the relevant antigen. No agglutination constitutes a negative test result and indicates that the antigen is not present.

Reagents:

Gamma-clone Anti-Fy^b (Monoclonal) Blood Grouping Reagent is prepared from human IgM antibodies from the hybridoma cell line SpA264LBg1 grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Sodium azide is added as a preservative (at 0.09% w/v). Ready for use as supplied.

Any Bovine Albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This ruminant-based product is deemed to have a low-TSE (Transmissible Spongiform Encephalopathy) risk.

Storage:

- Store at 1ºC to 10ºC when not in use.
- Do not use beyond the expiration date which is expressed as CCYY-MM-DD (year-month-date).
- Do not freeze.

Precautions:

- For *in vitro* diagnostic use.
- Do not dilute.
- Effort should be made to minimize contamination during use.
- Do not use if markedly turbid.

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Sodium azide is added as a preservative (at 0.09% w/v). Waste fluids arising from the use of Gamma-clone Anti-Fy^a (Monoclonal) must be flushed with large quantities of water to avoid accumulation of potentially explosive compounds in laboratory plumbing.

Handle and dispose of reagent as potentially infectious.

Specimen Collection and Preparation:

No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique, with or without an anticoagulant. Samples drawn into EDTA, ACD, CPD, CP2D and CPDA-1, as well as red blood cells that have been stored in the additive solutions AS-1, AS-3 and AS-5 can be used for testing. The specimen should be tested as soon as possible after collection. If delay in testing should occur, the specimen must be stored at 1°C to 10°C. Bacterial contamination of the specimen may cause false test results. Blood drawn into EDTA should not be stored for longer than ten days. Clotted specimens may be tested up to 21 days after collection, and donor blood may be tested up to the expiration date. Storage may result in weaker-than-normal reactions.

Procedure:

Materials Provided:

Gamma-clone Anti-Fy^b (Monoclonal)

Materials Required But Not Provided:

1. Test tubes (12x75 mm or 10x75 mm)
2. Pipettes
3. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5
4. Timer*
5. Centrifuge*
6. An optical aid such as a hand lens or concave mirror
7. Red blood cells of known Duffy phenotypes for use as controls.

*It is the users responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method:

1. Place one (1) drop of Gamma-clone Anti-Fy^b (Monoclonal) into a properly labeled test tube.
2. Add one (1) drop of an approximate 2-5% suspension of the red blood cells to be tested to the test tube (from step 1 above). The red blood cells to be tested must previously have been washed at least one time and then resuspended in saline.
3. Mix the test tube contents well by gently shaking the tube.
4. Incubate the test tube from five (5) to fifteen (15) minutes at room temperature (15°C to 30°C). Incubating at the upper end of the time range may enhance reactivity.
5. Centrifuge the test tube for:
   (a) one (1) minute between 100 and 125 xg, or
   (b) fifteen (15) seconds between 900 and 1,000 xg, or
   (c) a time and speed appropriate to the calibration of the centrifuge.
6. After centrifugation, immediately resuspend the red blood cells by gently shaking the test tube and examine for macroscopic agglutination. Negative reactions may be examined with an optical aid; however, microscopic reading is not recommended. Record the results.

Stability of Reaction:

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative or, at most, weakly positive reactions.

Quality Control:

The reactivity of blood grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigens. Fy(a+b+) red blood cells are the most suitable positive control red blood cells for Anti-Fy^b. Immucor Reagent Red Blood Cells are a convenient source of control cells and may be used as supplied. The reagent can be considered to be satisfactory for use if it reacts suitably with known Fy^b antigen positive and negative red blood cells.

Spontaneous agglutination of the red blood cells does not commonly cause false test results when blood grouping tests are carried out, especially when the red blood cells are washed before testing. If desired, however, a control test for spontaneous agglutination, using

Monoclonal Control in place of the reagent, may be carried out on any red blood cell suspension showing a positive reaction with the reagent. This control is not required if the specimen has already been tested and found to show no spontaneous agglutination.

Interpretation of Results:

Agglutination of the red blood cells constitutes a positive test result and indicates the presence of the Fy^b antigen.

No agglutination constitutes a negative test result, and indicates the absence of the Fy^b antigen.

The reaction patterns possible with Anti-Fy^a and Anti-Fy^b are shown in Table 1, together with the frequencies of the resulting phenotypes in some ethnic populations.

Reagent		Phenotype	Frequency (%) [5]	
Anti-Fy^a	Anti-Fy^b		Caucasian	Blacks
+	0	Fy(a+b–)	17	9
+	+	Fy(a+b+)	49	1
0	+	Fy(a–b+)	34	22
0	0	Fy(a–b–)	Very rare	68

Table 1: The reaction patterns of Anti-Fy^a and Anti-Fy^b, and the approximate frequencies of the resulting phenotypes in some ethnic populations.

Limitations:

1. Factors that may cause false test results include the following:
   a. Bacterial or chemical contamination of blood specimens, reagent and/or supplementary materials.
   b. Improper storage of materials.
   c. Aged or stored blood specimens, which may yield weaker reactions than those obtained with fresh red blood cells.
   d. Too heavy a red blood cell suspension of the specimen.
   e. Improper incubation time or temperature.
   f. Improper centrifugation. Proper centrifugation calibration is particularly important to the proper performance of the test. Excessive centrifugation may lead to difficulty in resuspending the red blood cell button in the tube test leading to a possible false positive result. At the same time, inadequate centrifugation may yield unclear red blood cell button patterns and agglutinates that are too readily dispersed leading to a possible false negative result.
   g. Improper examination for agglutination (usually too vigorous shaking). The resuspension of reactions in the tube test procedure must be carried out by gentle shaking. Shaking too vigorously may cause agglutinates to be dispersed.
   h. Deviation from the recommended test procedure such as the omission of test reagents.
2. Red blood cells that have been enzyme-treated must not be used for testing as either red blood cells under investigation or as a source of control red blood cells because use of these enzyme-treated red blood cells may yield erroneous results.

Specific Performance Characteristics:

Gamma-clone Anti-Fy^b (Monoclonal) meets FDA potency requirements. Each lot is tested by insert methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity and specificity. The specificity of the monoclonal antibodies secreted by the cell line used to manufacture this Blood Grouping Reagent has been determined by testing with red blood cells of varying phenotypes.

The performance of this product is dependent upon adhering to the package insert recommended methodology.

Performance Characteristics by manual tube method:

Method comparison studies were performed at three (3) external sites and one (1) internal site. Immucor, Inc., as the manufacturer, was the internal site. The external sites were representative of blood collection establishments, hospital-based transfusion services, and/or clinical laboratories. The sites were selected to capture a diverse sample population based on geographic location and facility demographics. Specimens were tested with the reagent and also a comparator reagent. Test results were evaluated for agreement between reagents. Combined results from all sites are summarized in the following table:

Note: Agreement between methods does not indicate which method is correct.

N=1799		Comparator Reagent†		[redacted]	
		Positive	Negative		
Anti-Fy^b	Positive	1258	6	Positive Percent Agreement	100.00%
				PPA (95% 1-Sided LCI)	99.76%
	Negative	0[1]	535	Negative Percent Agreement	98.89%
				NPA (95% 1-Sided LCI)	97.60%

†The comparator reagent does not claim to detect the Fy^x phenotype. Discordant samples were further genotyped by DNA molecular testing (PreciseType™ HEA BeadChip). All six discordant samples agreed with the Gamma-clone Anti-Fy^b results. All six samples were GATA negative.One sample typed Fy(a+b+) and the remaining 5 samples typed as Fy(a+b+^{w}) [Fy_{mod} (Fy^x)] due to the 265C>T SNP. [1]One sample demonstrating the Fy(a+b+^{w}) (FY_{mod} (Fy^x) phenotype due to the 265C>T SNP initially tested Fy(b+) and Fy(b-) upon retest. The table utilizes the initial result of positive. Resolved NPA 100.00% (99.44% 95% 1-sided LCI).

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

Bibliography:

1. Cutbush M, Mollison PL, Parkin DM. A new human blood group. Nature 1950; 165:188.
2. Ikin EW, Mourant AE, Pettenkofer HJ, Blumenthal G. Discovery of the expected haemagglutinin, anti-Fy^b. Nature 1951; 168:1077.
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4. Chown B, Lewis M, Kaita H. The Duffy blood group system in Caucasians: evidence for a new allele. Am J Hum Genet 1965; 17:384-389.
5. Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen facts book. 3rd ed. San Diego: Elsevier Academic Press, 2012:365.

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