BLOOD GROUPING REAGENT

Anti-Fy^a (Monoclonal)
Gamma-clone®

By Indirect Antiglobulin Test

Preservative: <0.1% Sodium Azide 1°C 10°C Meets FDA Potency Requirements IVD

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IMMUCOR GAMMA®

Intended Use:

Gamma-clone Anti-Fy^a (Monoclonal) Blood Grouping Reagent is intended for the detection of the Fy^a (FY1) antigen on red blood cells by the indirect antiglobulin 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^a antigen is determined by testing with Anti-Fy^a by the indirect antiglobulin 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^a (Monoclonal) Blood Grouping Reagent is prepared from IgG antibodies from the human/murine heterohybridoma cell line P3TIM 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 less than 0.1% 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 US Veterinary 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.

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.

Sodium azide is added as a preservative (at less than 0.1% 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^a (Monoclonal)

Additional Materials Required:

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. 37°C waterbath or incubator*
5. Timer*
6. Centrifuge*
7. Automated test tube washing device for use with antiglobulin test* (optional)
8. An optical aid such as a hand lens or concave mirror
9. Anti-Human Globulin containing anti-IgG
10. IgG-sensitized red blood cells
11. 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^a (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 and incubate the tube from ten (10) to fifteen (15) minutes at 36°C to 38°C.
4. Wash the tube contents a minimum of three (3) times with saline, being careful to decant the saline between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash. Decant the saline completely following the last wash.
5. Add one (1) or two (2) drops of Gamma-clone® Anti-Human Globulin (Anti-IgG or Anti-IgG,-C3d; Polyspecific) to the washed button of red blood cells, or follow the directions of the Anti-Human Globulin (AHG) manufacturer.
6. Mix the test tube contents well by gently shaking the tube and centrifuge the 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.
7. 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:

The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.

Quality Control:

1. All negative tests should be confirmed by adding IgG-sensitized red blood cells, such as Checkcell®, and then repeating centrifugation and reading. A positive test result at this point confirms that active antiglobulin (anti-IgG) was added to the test system and was present when the original test was interpreted as negative.

2. 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^a. Immucor Reagent Red Blood Cells are a convenient source of control cells and may be used as supplied.
3. It is necessary to carry out a direct antiglobulin test on each red blood cell suspension being typed, to confirm that any agglutination is truly due to an antigen-antibody reaction between the test red blood cells and the blood grouping reagent. This control may be omitted if the tests are negative or if the red blood cells are being typed by the indirect antiglobulin technique with blood grouping reagents of other specificities and yield a negative result.

Interpretation of Results:

Agglutination of the red blood cells constitutes a positive test result and indicates the presence of the relevant antigen, providing the test red blood cells do not have a positive direct antiglobulin test.

No agglutination constitutes a negative test result, and indicates the absence of the relevant 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 having a positive direct antiglobulin test due to coating of IgG cannot be typed by the indirect antiglobulin technique.
3. 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^a (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 murine 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.

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.
3. Sanger R, Race RR, Jack JJ. The Duffy blood groups of New York Negroes: the phenotype Fy(a–b–). Brit J Haemat 1955; 1:370-374.
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. The blood group antigen facts book. 2nd ed. San Diego: Elsevier Academic Press, 2004:280.