BLOOD GROUPING REAGENT %

Anti-Fy^{a} and Anti-Fy^{b} %

(Murine Monoclonal) (Recombinant) %

Direct Agglutination by Tube Method

For *In Vitro* Diagnostic Use.
Preservatives: 0.1% sodium azide.
Handle and dispose of as if potentially infectious.
The absence of murine virus has not been determined.
Meets FDA potency requirements. '
Store at 2-8˚C. '
These Products Contain Dry Natural % **Rubber**.
Do not use if product is turbid.

INTENDED USE:

Siwa Biotech's blood grouping reagents, Anti-Fy^{a} and Anti-Fy^{b}, are for the detection of the corresponding red blood cell antigens when tested by direct agglutination by the tube method.

SUMMARY AND EXPLANATION:

The Duffy blood group system was discovered in 1950 when the first example of Anti-Fy^{a} was found in the serum of a multiply transfused patient. One year later the antithetical antigen Fy^{b} was identified. An important minor Duffy variant is the FYX allele that was originally described in 1965. The FYX allele encodes the Fy^{b} antigen, but it is only weakly expressed on the red cell surface, and it is not always detected by Anti-Fy^{b}.

Antibodies of the Duffy system are usually immune stimulated, IgG in nature and can bind complement. Anti-Fy^{a} and Anti-Fy^{b} have been implicated in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. The prevalence of common Duffy phenotypes is shown in the following table:

Phenotype	White %	Black %
Fy(a+b-)	17	9
Fy(a+b+)	49	1
Fy(a-b+)	34	22
Fy(a-b-)	Very rare	68

PRINCIPLE OF THE TEST:

The presence or absence of the Fy^{a} and Fy^{b} antigens is determined by testing red cells with Anti-Fy^{a} and Anti-Fy^{b} blood grouping reagent when tested by direct agglutination by the tube method. Following centrifugation of red cells and Anti-Fy^{a} and Anti-Fy^{b} reagents, the presence of the corresponding antigen is indicated by agglutination of the red cells. Lack of agglutination is interpreted as a negative test and indicates the absence of the corresponding antigen.

REAGENTS:

Siwa's Anti-Fy^{a} (FY1) is a recombinant murine IgM monoclonal antibody expressed from Chinese Hamster Ovary (CHO) cell line 310 that recognizes human Fy^{a}.

Siwa's Anti-Fy^{b} (FY2) is a recombinant murine IgM monoclonal antibody expressed from Chinese Hamster Ovary (CHO) cell line 401 that recognizes human Fy^{b}. The reagents display the specificity and reproducibility typically associated with monoclonal antibodies and meet FDA potency requirements. The antibodies are diluted in a buffered solution containing bovine albumin, macromolecular potentiators and sodium azide as a preservative. The expiration date format is expressed as YYYY-MM-DD.

WARNINGS AND PRECAUTIONS:

1. These reagents should be handled and disposed of as if potentially infectious. The absence of murine virus has not been determined.
2. ' **These Products Contain Dry Natural Rubber.**
3. ' Do not use if product is turbid.
4. ' Sodium azide is added as a preservative at 0.1% (w/v). Sodium azide is toxic and harmful if swallowed. An accumulation of sodium azide may result in a reaction with lead or copper plumbing to form explosive metal azide complex. If the reagent is discarded into sinks, flush with large amounts of water to prevent azide build-up.
5. ' The bovine albumin used in the formulation of these products is obtained from a USDA approved Transmissible Spongiform Encephalopathies (TSE) free source.
6. ' For *In Vitro* Diagnostic Use.

STORAGE:

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

SPECIMEN COLLECTION AND PREPARATION:

Samples should be collected using standard approved methods. The sample should be tested as soon as possible; however, samples may be stored at 2-8˚C if necessary. Blood drawn into EDTA, ACD, CPD, CPD with AS-1, CPD with AS-5, CPDA-1, CP2D, and CP2D with AS-3 can be used for testing. Samples drawn in EDTA should be tested within 7 days. Clotted samples may be tested up to 14 days. Donor blood may be tested until expiration of the unit. Blood samples showing gross hemolysis or contamination should not be tested. Storage of red cell samples may result in weakened reactions.

MATERIALS:

Materials provided

Siwa Biotech Monoclonal Anti-Fy^{a}
Siwa Biotech Monoclonal Anti-Fy^{b}

Materials required but not provided

Test tubes – 12 x 75 mm or 10 x 75 mm
Pipettes
Isotonic saline or phosphate buffered saline
Calibrated serofuge
Calibrated timer
Agglutination viewing mirror or equivalent

TEST PROCEDURE:

1. ' Perform testing at 18-25˚C.
2. ' Wash the red cell sample at least once with isotonic or phosphate buffered saline and prepare a 2-4% red cell suspension. Reagent red cells do not require washing.

3. ' Place one drop of the test reagent into a properly labeled test tube.
4. ' Add one drop of the red cell suspension. Mix by shaking the tube.
5. ' Centrifuge for 15 to 20 seconds at 900-1000 x g* or a time and speed appropriate for the centrifuge used.
6. ' Resuspend the red blood cells by gently shaking the test tube and immediately examine for macroscopic agglutination. Record results.
   Stability of Reaction: Test results must be interpreted immediately upon completion of the test. Delays in interpreting the results may result in weakened reactivity.

* The time and speed of centrifugation used should be sufficient to produce the strongest reaction of antibody with antigen-positive red blood cells, and yet permit easy resuspension of antigen-negative red blood cells.

INTERPRETATION OF RESULTS:

Positive test: Agglutination of red cells.** '
Negative test: No agglutination of red cells. '
** Siwa Anti-Fy^b detects some Fy^x (Fy^b weak) samples that may ' previously or currently type as Fy(b-) using other licensed Anti-Fy^b ' blood grouping reagents (BGR). '

QUALITY CONTROL:

Controls of known antigen positive and negative red cells, preferably those exhibiting a heterozygous expression of the test antigen, should be tested at least daily. Tests must be considered invalid if controls do not show the expected reactions.

LIMITATIONS:

1. ' Red cells that have been treated with proteolytic enzymes are not suitable for testing and may produce falsely positive or negative results.
2. ' Too heavy of a red cell suspension may result in weakened reactivity.
3. ' Proper centrifuge calibration is critical. Excessive centrifugation can result in difficulty in resuspending red cell buttons and insufficient centrifugation can cause weak agglutinates that can be too readily dispersed.
4. ' Shaking the red cell button too vigorously may cause weakened reactivity.
5. ' Erroneous results may occur due to improper storage of test sample and reagents, contamination, improper centrifugation, and failure to follow the Test Procedure.
6. ' Interfering Substances – Blood samples showing gross hemolysis or contamination should not be tested and may produce falsely positive or negative results.

SPECIFIC PERFORMANCE CHARACTERISTICS:

Siwa Biotech's blood grouping reagents Anti-Fy^a and Anti-Fy^b, are manufactured to meet FDA potency requirements. Each lot is tested against a panel of antigen positive and negative cells to ensure suitable reactivity and specificity when tested by the Test Procedure. The specificity of the monoclonal antibodies was established by testing with blood cells of varying phenotypes. The performance of the reagents was confirmed against FDA-licensed blood grouping reagents in a comparison study in which the reagents were tested in parallel at different clinical sites. The percent agreements and one-sided 95% lower confidence limits for all sites combined are shown in the tables below.

Table 1. Comparison Study Results for Anti-Fy^a

		Comparator BGR		
		Positive	Negative	
Siwa BGR	Positive	804	1	805
	Negative	5	573	578
		809	574	1,383

	Agreement	95% LCL
PPA	99.4%	98.7% [1]
NPA	99.8%	99.2%

Table 2. Comparison Study Results for Anti-Fy^b

		Comparator BGR		
		Positive	Negative	
Siwa BGR	Positive	900	12	912
	Negative	5	469	474
		905	481	1,386

	Agreement	95% LCL
PPA	99.4%	98.8% [2]
NPA	97.5%	96.0% [2]

PPA = Positive Percent Agreement
NPA = Negative Percent Agreement
LCL = Lower Confidence Limits

1. Discordant results for Anti-Fy^a were resolved by testing with a licensed blood typing method and showed 3 of 5 Siwa BGR results to be concordant with the resolution test method.

2. Discordant results for Anti-Fy^b were resolved by testing with a licensed blood typing method and showed 9 of 13 Siwa BGR results to be concordant with the resolution test method. Six of the discordant samples were the result of Siwa BGR detecting Fy^x (Fy^b weak) samples that were not detected by the comparator BGR. Four of the discordant samples were ineligible for resolution testing.

For additional information or technical support, call Siwa Biotech toll free at 1-(844) 384-4306.

BIBLIOGRAPHY:

1. ' Roback, J.D., ed. Technical Manual, 17th ed. Bethesda, MD: AABB, 2011.
2. ' Reid, M.E., Lomas-Francis C., and Olsson M. The Blood Group Antigen FactsBook. 3rd ed. San Diego: Elsevier Academic Press, 2012.
3. ' Chown, B., Lewis, M., and Kaita, H. *Am. J. Hum. Gen*. 17(5):384-9, 1965.
4. ' Tournamille, C. et al, *Blood* 92(6):2147-56, 1998.

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