BLOOD GROUPING REAGENT

Anti-Fy^{a} (Monoclonal) (IgG)
Anti-Fy^{b}
ORTHO™ Sera

REF 6904546
6904547

MEETS FDA POTENCY REQUIREMENTS

INSTRUCTIONS FOR USE

Intended Use

For *in vitro* diagnostic use only
For use with the ID-Micro Typing System™ Anti-IgG Card
For Indirect Antiglobulin Test

The Anti-Fy^{a} reagent (Anti-FY1) is for the qualitative *in vitro* detection of human Fy^{a} positive red blood cells by the indirect antiglobulin test.
The Anti-Fy^{b} reagent (Anti-FY2) is for the qualitative *in vitro* detection of human Fy^{b} positive red blood cells by the indirect antiglobulin test.

Summary and Explanation

Anti-Fy^{a} and anti-Fy^{b} (Anti-FY1 and Anti-FY2) were described in 1950 and 1951 respectively. Fy^{a} and Fy^{b} are a pair of alleles on the long arm of chromosome 1, giving rise to three commonly encountered phenotypes: Fy(a+b-), Fy(a+b+) and Fy(a-b+). Fy^{a} and Fy^{b} antigens are destroyed when the red blood cells are treated with appropriate concentrations of the proteolytic enzymes ficin, papain, and α-chymotrypsin.[1]

Principles of Procedure

When used by the recommended technique, the reagents will cause agglutination (clumping) of red blood cells carrying the Fy^{a} or Fy^{b} antigen. Lack of agglutination of the red blood cells demonstrates the absence of the Fy^{a} or Fy^{b} antigen.

Reagents

Anti-Fy^{a} (Monoclonal) (IgG) and Anti-Fy^{b} are supplied as two separate reagents.

Anti-Fy^{a} (Monoclonal) (IgG) is supplied as one reagent.

- 1 vial containing 5 mL of human monoclonal antibodies of type IgG (cell line DG-FYA-02) containing <0.1% (w/v) sodium azide and potentiators.

Anti-Fy^{b} is supplied as one reagent.

- 1 vial containing 5 mL of human polyclonal antibodies of type IgG containing 0.1% (w/v) sodium azide and bovine material (i.e., bovine serum albumin, fetal bovine serum).

Any bovine material used in the manufacture of these products is sourced from USDA approved facilities.

No preparation of the reagent(s) is required. Use directly from the vial. Do not dilute.

Storage Requirements

Store at 2–8 °C.
Do not freeze.
Do not use beyond expiration date. The format of the expiration date is expressed as YYYY-MM-DD (year-month-day).
May be at 18–25 °C while in use.
Replace cap when not in use.

Specimen Collection

- No special preparation of the patient/donor is required prior to specimen collection.
- Specimens should be collected by aseptic technique with an anticoagulant.
- The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at 2–8 °C.
- Do not use collection tubes that contain plasma/cell separation media.
- When using ORTHO™ Sera Anti-Fy^{b} do not use samples collected from cord blood
- Samples collected in EDTA should be tested within seven days from collection.
- Donor blood collected in ACD, CPD, CP2D, CP2D with AS-3 and CPDA-1may be tested until the expiration date of the donation.

NOTE: ORTHO™ Sera Anti-Fy^{a} (Monoclonal)(IgG) has not been validated for use with CP2D with AS-3. Do not use ORTHO™ Sera Anti-Fy^{a} with this anti-coagulant/additive solution.

- Clotted, hemolyzed, grossly icteric or contaminated blood specimens should not be used.
- Grossly lipemic samples containing particulates that clog the gel, as indicated by diffuse blotches of red blood cells in the microtube, may be clarified by centrifugation or filtration and retested.
- Specimens should not be exposed to extreme heat.

Precautions

Do not use if turbid.
Do not dilute.
Do not freeze.
Do not use beyond the expiration date.
The Anti-Fy^{a} reagent contains < 0.1% (w/v) sodium azide.
The Anti-Fy^{b} reagent contains 0.1% (w/v) sodium azide.
Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.
This reagent is for *in vitro* diagnostic use only.

CAUTION:	**Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide buildup.**
CAUTION:	**Source material from which this product is derived was found non-reactive for HBsAg, Anti-HIV 1/2 and Anti-HCV. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious disease. Appropriate care should be taken in the use and disposal of this product. Source materials may include human components and antibody producing cells that are used in the manufacture of polyclonal and monoclonal products.**

Procedure

Materials Provided

ORTHO™ Sera Anti-Fy^{a}
ORTHO™ Sera Anti-Fy^{b}

Materials Required but not Provided

- Isotonic saline
- Reagent red blood cells suitable for the control of Anti-Fy^{a}
- Reagent red blood cells suitable for the control of Anti-Fy^{b}
- MTS™ Anti-Human Globulin Anti-IgG Card

NOTE:	Store cards upright at 2–25 °C.
CAUTION:	**Inspect the condition of the card before use.** **Do not use gel cards that have not been shipped in an upright position.** **Do not use cards beyond expiration date.** **Do not freeze or expose cards to excessive heat.** **Use reagents as furnished.**

- Micropipetters for delivery of 25 µL and 50 µL
- Pipet tips
- Marking pen
- MTS™ Centrifuge and MTS™ Incubator
- ORTHO™ Workstation

Test Procedure

NOTE:

The reagents have been standardized for use by the technique described below.

The indirect agglutination test procedure listed below is for manual testing only. When using supplemental testing equipment (i.e., incubator, centrifuge), follow the procedures that are contained in the operator's manual provided by the device manufacturer.

Indirect Antiglobulin Test

1. Prepare an approximate 0.8% red blood cell suspension from patient or donor cells, using isotonic saline.

2. Allow the card and the reagent(s) to come to 18–25 °C before use. A clear liquid layer should appear on top of the opaque gel in each microtube.
3. Visually inspect gel cards before use.

CAUTION:	**Do not use gel cards if the gel matrix is absent or the liquid level in the microtube is at or below the top of the gel matrix.** **Do not use gel cards that show signs of drying, discoloration, bubbles, crystals, or other artifacts.** **Do not use cards if foil seals appear damaged or opened.**

NOTE:	Refer to the ID-Micro Typing System™ Interpretation Guide[2] for additional information related to the visual inspection of gel cards before use.

4. Label the card appropriately with a sample identifier.
5. Remove the foil seal from the MTS™ Anti-IgG Card or from the individual microtubes to be used for testing.

CAUTION:	**Do not remove card foil seal until ready to use. Foil should be removed immediately before testing or within 1 hour of testing. Once opened, the gel may begin to dry out which could affect test results (refer to Limitations of the Procedure). After removing the foil, visually inspect all gel cards to ensure that residual film does not block the opening of any microtube.**

6. Add 25 µL of the reagent to the appropriate reaction chamber(s) of the opened card.

CAUTION:	**Do not touch the pipet to the side of the reaction chamber. If this occurs, change the pipet tip before proceeding to the next chamber.**

7. Add 50 µL of 0.8% red blood cell suspension to the appropriate reaction chamber(s) of the card.

CAUTION:	**Do not touch the pipet to the side of the reaction chamber. If this occurs, change the pipet tip before proceeding to the next chamber.**

8. Observe that the contents of the reaction chamber(s) are combined. If necessary tap gently.

NOTE:	Assure that the reagents remain in the reaction chamber. There should be no mixing of reactants with reagents in the column prior to centrifugation.

9. Incubate at 37 °C ± 2 °C for 15 minutes.
10. Centrifuge the card at the preset conditions, as installed by the instrument manufacturer.
11. Read the front and back of the individual columns for macroscopic agglutination or hemolysis upon test completion.
12. Record the reaction strength.

Interpretation of Results

Negative Result = No agglutination and no hemolysis of the red blood cells is a negative test result. A complete sedimentation of all red blood cells is present in the bottom of the microtube.

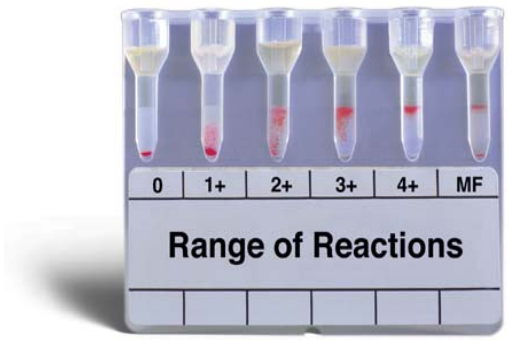
Positive Result = Agglutination of the red blood cells is a positive test result. Red blood cells may remain suspended on the top of the gel or are dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions.

Reaction Grading Guide (Use in conjunction with Diagram 1)	
0 Negative	Unagglutinated red blood cells form a well-defined button at the bottom of the microtube.
1+ Reaction	Red blood cell agglutinates are observed predominantly in the lower half of the gel microtube. Unagglutinated red blood cells form a button in the bottom of the microtube.
2+ Reaction	Red blood cell agglutinates are dispersed throughout the length of the gel microtube. Few unagglutinated red blood cells may be observed in the bottom of the microtube.
3+ Reaction	The majority of red blood cell agglutinates are trapped in the upper half of the gel microtube.
4+ Reaction	Solid band of red blood cell agglutinates on top of the gel. A few agglutinates may filter into the gel but remain near the predominant band.
Mixed Field	Red blood cell agglutinates at the top of the gel or dispersed throughout the gel microtube accompanied by a button of negative red blood cells in the bottom of the microtube. See Note below.

NOTE: Caution must be taken in interpreting a reaction as mixed field. Additional patient history and testing will be necessary for resolution. However, not all mixed cell situations have a sufficient minor population to be detected.

CAUTION: **Clots, particulates or other artifacts may cause some red blood cells to be entrapped at the top of the gel that may cause an anomalous result in a negative test (refer to Limitations of the Procedure, item 6.)**

Diagram 1: Examples of Reaction Grades



NOTE: Refer to ID-Micro Typing System™ Interpretation Guide[2] for additional information.

Stability of Reaction

For best results, it is recommended that reactions should be read immediately following centrifugation.

Quality Control

Quality Control (QC) of reagents is required. Quality Control should be performed on each lot of reagent on each day of use according to standard operating procedures.

Limitations of the Procedure

1. Strict adherence to the procedures and use of recommended equipment is essential.
2. Proper incubator parameters are important to the performance of the MTS™ Anti-IgG Card. The MTS™ Incubator and ORTHO™ Workstation have been exclusively designed to provide the correct parameters for time and temperature.
3. Proper centrifuge calibration is particularly important to the performance of the MTS™ Anti-IgG Card. The MTS™ Centrifuge and ORTHO™ Workstation have been exclusively designed to provide the correct time, speed and angle.
4. The expression of certain red blood cell antigens may diminish in strength during storage, particularly in EDTA samples. Better results will be obtained with fresh samples.
5. Suppressed or weak expression of blood group antigens may give rise to false-negative reactions.
6. Anomalous results may be caused by the following:
   - Fibrin or particulate matter.

- Red blood cell samples from cord blood samples when using ORTHO™ Sera Anti-Fy^{b}.
- Red blood cells sticking to the sides of the reaction chamber.
- Red blood cell samples from patients with Sickle Cell Disease and patients with Malignancy, where there may be a history of previous transfusion.
- DAT positive red blood cells.
- Do not use cards that appear damaged (i.e., break in foil seal or break, crack or bubble in the column), exhibit drying (i.e., liquid level is at or below the top of the gel matrix) or exhibit discoloration (due to bacterial contamination, which can cause false reactions).
- Loss of fluid in the card column may cause (weak) false positive results.
- J reactions may occasionally be observed with high red blood cell concentrations. J reactions may also be observed if during centrifugation the card is not seated properly in the holder or not allowed to spin at a 90° angle.

  NOTE: A J reaction consists of cells forming a button at the bottom of the gel matrix or microtube when either end of the cell button goes up the side of the column. The cell button may be disrupted. A J reaction may represent a weakly positive reaction.
- False positive or false negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test samples.

7. Tests with these or other anomalous results should be repeated.
8. Erroneous results could occur if final reactions are not read upon completion of centrifugation.
9. Mixed cell populations may be encountered as a result of, for example, transfusion, fetal maternal hemorrhage, or transplantation. Consult patient history when results of this nature are encountered before assigning an antigen type.
10. Red blood cells must be diluted with isotonic saline, before adding the red blood cells to the microtube.

Performance Characteristics

Comparator Study Results

During comparator studies (data on file at Alba Bioscience Limited), blood samples were tested with ORTHO™ Sera Anti-Fy^{a} (Monoclonal)(IgG) and ORTHO™ Sera Anti-Fy^{b} by ORTHO ID-Micro Typing System™ Column Agglutination Technology (CAT) as follows:

Including all samples:

Test	Positive			Negative		
	N	% Agreement*	One-Sided Exact 95% LCL (%)	N	% Agreement*	One-Sided Exact 95% LCL (%)
Anti-Fy^{a}	738	100.0	99.6	380	97.4	95.6
Anti-Fy^{b}	782	99.7	99.2	353	93.5	90.9

Excluding DAT positive samples:

Test	Positive			Negative		
	N	% Agreement*	One-Sided Exact 95% LCL (%)	N	% Agreement*	One-Sided Exact 95% LCL (%)
Anti-Fy^{a}	738	100.0	99.6	374	98.9	97.6
Anti-Fy^{b}	782	99.7	99.2	348	94.8	92.4

LCL: lower confidence limit

* % Agreement between the ORTHO™ Sera Anti-Fy^{a} (Monoclonal)(IgG), ORTHO™ Sera Anti-Fy^{b} and comparator reagents only and does not indicate which reagents gave the correct results.

Anti-Fy^{a}

In performance evaluation studies, 1118 samples were tested with ORTHO™ Sera Anti-Fy^{a} (Monoclonal) (IgG) using the MTS™ Incubator and MTS™ Centrifuge. The one-sided exact 95% LCL of positive percent agreement was ≥99% for agglutination tests based on a comparison of interpreted results. The one-sided exact 95% LCL of negative percent agreement (NPA) was 95.6% for agglutination tests based on a comparison of interpreted results. The NPA did not meet the acceptance criteria due to 10 discrepant results (see sample classification and comments in the summary table below).

The discordance between the trial and the comparator reagent could be attributed in six cases to the sample itself having a positive DAT result and in two cases, where a change from the initial test outcome was noted, to a possible error with the initial comparator test result and the differences in sensitivity between the trial and comparator regent test methods (Weisbach et al., 1999; Weisbach et al., 2006). In the remaining two cases, where the initial test result was confirmed on investigation, no definitive root cause could be determined for the discrepant results. For these remaining two cases, both samples are included in the classification 'Sickle Cell Disease and patients with Malignancy'.

Classification	Number of Discrepancies	Comment
DAT Positive	6	Reagents which use an IAT method are not recommended for testing of samples with a positive DAT.
Sickle Cell Disease and patients with Malignancy	2	Care should be taken when interpreting results, particularly where there may be a history of previous transfusion.
Possible test error	2	ORTHO™ Sera Anti-Fy^{a} reagent and comparator reagent gave a concordant reaction on repeat testing.

Anti-Fy^{b}

In performance evaluation studies, 1135 samples were tested with ORTHO™ Sera Anti-Fy^{b} using the MTS™ Incubator and MTS™ Centrifuge. The one-sided exact 95% LCL of positive percent agreement was ≥99% for agglutination tests based on a comparison of interpreted results. The one-sided exact 95% LCL of negative percent agreement (NPA) was 90.9% for agglutination tests based on a comparison of interpreted results. The NPA did not meet the acceptance criteria due to 25 discrepant results (see sample classification and comments in the summary table below).

The discordance between the trial and the comparator reagent could be attributed in five cases to the sample itself having a positive DAT result, in four cases to the inability of the comparator reagent to detect the Fy^{x} phenotype and in seven cases, where a change to the initial test outcome was noted, to a possible error with the initial comparator test result. In the remaining nine cases, where the initial test result was confirmed on investigation, no definitive root cause could be determined for the discrepant results. For these remaining nine cases, two samples are included in the classification 'Sickle Cell Disease and patients with Malignancy', six samples are from cord blood and one sample has no specific classification (see Unresolved in the summary table below).

Discrepant test results were noted with cord blood samples which could not be attributed to sample condition, therefore, please see the Limitations of the Procedure Section for further advice.

Classification	Number of Discrepancies	Comment
DAT Positive	5	Reagents which use an IAT method are not recommended for testing of samples with a positive DAT.
Weak antigen expression	4	ORTHO™ Sera Anti-Fy^{b} reagent showed reactivity against four known examples of Fy^{x} antigen.
Possible test error	7	ORTHO™ Sera Anti-Fy^{b} reagent and comparator reagent gave a concordant reaction on repeat testing.
Sickle Cell Disease and patients with Malignancy	2	Care should be taken when interpreting results, particularly where there may be a history of previous transfusion.
Cord	6	ORTHO™ Sera Anti-Fy^{b} reagent gave a positive reaction when the comparator reagent was negative.
Unresolved	1	ORTHO™ Sera Anti-Fy^{b} reagent and comparator reagent continued to show different result following repeat testing.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

Further migration studies have been performed using the ORTHO™ Workstation and results were as follows:

Reagent	Number of samples tested	Concordance*	Positive Samples	
			N	Frequency (%)
Anti-Fy^{a}	100	100.0%	60	60
Anti-Fy^{b}	100	100.0%	77	77

*Concordance indicates agreement between the ORTHO™ Workstation and the MTS™ Incubator/MTS™ Centrifuge only and does not indicate which systems gave the correct results.

Precision Study Results

As part of the performance evaluation, precision and lot to lot studies were performed using multiple operators, days and runs to confirm repeatability and reproducibility of test results in the same run, day and with the same operator and between runs, days and operators. The study took account of variables such as days of the week, times of day and supplementary reagents used in the testing. There were no discordant results; all expected positive test outcomes generated unequivocal positive reactions and all expected negative test outcomes generated unequivocal negative reactions.

Specific Performance Characteristics

Prior to release, each lot of ORTHO™ Sera Anti-Fy^{a} (Monoclonal)(IgG) and ORTHO™ Sera Anti-Fy^{b} are tested in alignment with FDA recommendations against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity.

ORTHO™ Sera Anti-Fy^{a} (Monoclonal)(IgG) and ORTHO™ Sera Anti-Fy^{b} Blood Grouping Reagents have been tested using the ID-Micro Typing System™ and when stored and used according to the recommended instructions for use, found to specifically agglutinate human red blood cells with the corresponding antigen.

The ORTHO™ Sera Anti-Fy^{a} (Monoclonal)(IgG) reagent reacts with cells expressing the Fy^{a} antigen and meets FDA potency requirements.

The ORTHO™ Sera Anti-Fy^{b} reagent reacts with cells expressing the Fy^{b} antigen and meets FDA potency requirements.

For additional information or technical support, contact OCD Customer Technical Support on 1-800-421-3311.

Bibliography

1. Reid, M.E., Lomas-Francis, C. and Olsson, M.L. (2012). The Blood Group Antigen Facts Book, 3rd ed. London, Academic Press.
2. ID-Micro Typing System™ Interpretation Guide (J6902201), Ortho Clinical Diagnostics.
3. Weisbach, V., Ziener, A., Zimmermann, R., Glaser, A., Zingsem, J. and Eckstein, R. (1999) 'Comparison of the performance of four microtube column agglutination systems in the detection of red cell alloantibodies', *Transfusion*, 39(10), pp. 1045-1050.
4. Weisbach, V., Kohnhauser, T., Zimmermann, R., Ringwald, J., Strasser, E., Zingsem, J. and Eckstein, R. (2006) 'Comparison of the performance of microtube column systems and solid-phase systems and the tube low-ionic-strength solution additive indirect antoglobulin test in the detection of red cell alloantibodies', *Transfusion Medicine*, 16(4), pp. 276-284.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Do Not Reuse	Contains Sufficient for "n" Tests	Fragile, Handle with Care.
Use by or Expiration Date (Year-Month-Day)	*In vitro* Diagnostic Medical Device	Keep Dry
LOT Batch Code or Lot Number	Upper Limit of Temperature	This end up
SN Serial Number	Lower Limit of Temperature	Do Not Use if Damaged
REF Catalog Number or Product Code	Temperature Limitation	Card
Caution	Consult instructions for use	CONC Concentration
Date of Manufacturer	Biological Risks	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations.
Manufacturer	Serious Health Hazards	
EC REP Authorized Representative in the European Community		

Summary of Revisions

Date of Revision	Version	Section	Description of Technical Changes*
2016-07-12	1.0		Initial version of Instructions for Use.

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

U.S. License No. 1807

Alba Bioscience Limited
Ellen's Glen Road, Edinburgh EH17 7QT, UK

Distributed by:

Ortho-Clinical Diagnostics, Inc.
1001 US Highway 202
Raritan, NJ 08869 USA

Ortho Clinical Diagnostics