**INTENDED USE**

BIOSCOT Anti-Fy<sup>a</sup> is a monoclonal human IgG blood grouping reagent (cell line P3TIM) which will detect the Fy<sup>a</sup> antigen when tested according to the Gel, Column and Indirect antiglobulin tube techniques. This reagent is designed for use by operators trained in serological techniques.

**INTRODUCTION**

Anti-Fy<sup>a</sup> was discovered in 1950 by Cutbush et al. Its antithetical partner, Anti-Fy<sup>b</sup>, was identified a year later by Ikin et al. The Fy<sup>a</sup> and Fy<sup>b</sup> genes produce 3 phenotypes: Fy(a+b-), Fy(a+b+) and Fy(a-b+). A third gene Fy<sup>d</sup> gives rise to a fourth phenotype Fy(a-b-). Antibodies of the Duffy system can cause delayed transfusion reactions and haemolytic disease of the newborn.

The frequencies of the Duffy phenotypes vary widely in different populations:

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fy(a+b-)</td>
<td>17%</td>
<td>9%</td>
<td>91%</td>
</tr>
<tr>
<td>Fy(a+b+)</td>
<td>49%</td>
<td>1%</td>
<td>9%</td>
</tr>
<tr>
<td>Fy(a-b+)</td>
<td>34%</td>
<td>22%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Fy(a-b-)</td>
<td>0%</td>
<td>67.6%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**PRINCIPLE OF THE REAGENT**

When used by the recommended techniques this reagent will cause agglutination (clumping) of red cells carrying the specific antigen (positive test). Lack of agglutination of the red cells demonstrates the absence of the specific antigen (negative test).

This reagent has been optimised for use by the recommended techniques without further dilution or additions.

This reagent has been supplied filtered to 0.22 µm.

**MATERIALS**

Product code NW Anti-Fy<sup>a</sup> is composed of monoclonal human IgG antibodies (cell line P3TIM) in a buffer solution. This reagent contains 0.1% (w/v) sodium azide and bovine material. Each vial (5 mL) contains sufficient material for approximately 100 tests.

**PRECAUTIONS**

1. All blood products should be treated as potentially infectious. The human donor or the cell line used to produce the Anti-Fy<sup>a</sup> reagent has been tested and found to be negative for HIV 1+2, HBV and HCV. Care must be taken in the use and disposal of each container and its contents.
2. This reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead or copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.
3. This product should be clear. Turbidity may indicate bacterial contamination. This reagent should not be used if a precipitate, fibrin gel or particles are present.
4. This reagent is for professional in vitro diagnostic use only.

5. The source of bovine material is either USDA approved or from sources where origin information is available. The donor animals have been inspected and certified disease free and are deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk.
6. This product should be disposed of either by overnight immersion in disinfectants at appropriate concentrations or by autoclaving.

**ADVICE TO USERS**

It is recommended that a positive control and a negative control should be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show the expected reactions.

It is not required to use a reagent control in parallel with all tests using this reagent. Only in typing the red cells of patients known to have auto antibodies or protein abnormalities is the use of a reagent control such as BIOSCOT Monoclonal control (Product code: TT) recommended. This should be tested in parallel with the reagent.

This reagent has been characterised by the procedures recommended in these instructions for use, its suitability for use in other techniques must be determined by the user.

In the event of changes in the analytical performance of the device or damage to the packaging please contact the Quality Assurance department at Millipore (UK) Ltd.

**STORAGE**

Store the opened / unopened product at 2-8°C until the expiry date detailed on the product label.

Failure to store the product at the correct temperature, for example, storage at higher temperature or repeated freezing and thawing, may result in accelerated loss of reagent activity.

**SPECIMEN COLLECTION**

No special preparation of the patient is required prior to specimen collection. Blood should be collected by an approved phlebotomy technique in to EDTA or citrate anticoagulant. The specimen should be tested as soon as possible following collection. If a delay in testing should occur, store the specimen at 2-8°C. Specimens displaying gross haemolysis or microbial contamination should not be tested with this reagent. Failure to store the specimens at the correct temperature may result in false positive or false negative results.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- **Indirect Antiglobulin Technique:**
  - Test tube
  - Isotonic saline or Phosphate Buffered Saline (PBS)
  - 37°C incubator
  - Timer
  - Anti-Human Globulin reagent
  - IgG sensitized red cells (Coombs control cells)

- **Bio-Rad Gel Technique:**
  - Bio-Rad ID-Card Coombs Anti-IgG or LISS/Coombs Cards.
  - Isotonic saline, phosphate buffered saline (PBS) or ID-Diluent 2
  - Microtitre plates capable of delivering 10, 25 and 50 μL
  - 37°C incubator
  - Timer
  - Centrifuge suitable for Bio-Rad ID-Cards
  - Reader (optional)

- **Grifols Gel Technique:**
  - DG Gel Anti-IgG or Coombs Cards
  - Isotonic saline, phosphate buffered saline (PBS) or DG Gel Sol
  - Microtitre plates capable of delivering 10, 25 and 50 μL
  - Timer
  - 37°C incubator Centrifuge (suitable for Grifols Gel Cards)
  - Reader (Optional)
BioVue Column Technique:
- Ortho BioVue System Anti-IgG or Anti-Human Polyspecific Cassettes
- Isotonic saline, phosphate buffered saline (PBS) or Ortho 0.8% Red Cell Diluent
- Micropipettes capable of delivering 10, 40 and 50 μL
- Timer
- Centrifuge suitable for Ortho BioVue Cassettes
- Reader (optional)

RECOMMENDED TECHNIQUES

1. INDIRECT ANTIGLOBULIN TECHNIQUE
1.1 Wash red cells at least once and prepare a 3-5% suspension of test red cells in isotonic saline or PBS.
1.2 Add one drop (40 μL) of Anti-Fy reagent to an appropriately labelled test tube.
1.3 Add one drop (40 μL) of the suspension of test red cells.
1.4 Mix and incubate at 37°C for 15 minutes.
1.5 Wash cells once with Isotonic saline, thoroughly decanting the saline.
1.6 Add 2 drops (80 μL) of Anti-Human Globulin reagent, mix and centrifuge tests at 1000 rcf for 20 seconds.
1.7 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.
1.8 To confirm that negative results are valid, add IgG sensitised red cells (Coombs control cells), repeat centrifugation and examine for agglutination. If no agglutination is observed the test is invalid and should be repeated.

2. BIO-RAD GEL TECHNIQUE
2.1 Prepare a 3-5% suspension of test red cells in Isotonic saline or PBS or a 0.8% suspension in ID- Diluent 2.
2.2 Add 10 μL of 3-5% or 50 μL of 0.8% suspension of test red cells to the appropriate microtube of the Bio-Rad ID-card.
2.3 Add 25 μL of Anti-Fy reagent to the appropriate microtube.
2.4 Mix gently and incubate at 37°C for 15 minutes.
2.5 Centrifuge the ID-card at appropriate time and speed according to card manufacturers instructions.
2.6 Read macroscopically or with a reader. The use of a reader must be validated by the user.

3. GRIFOLS GEL TECHNIQUE
3.1 Prepare a 3-5% or 1% suspension of test red cells in Isotonic saline or PBS or a 1% suspension in DG Gel Sol.
3.2 Add 10 μL of 3-5% or 50 μL of 1% suspension of test red cells to the appropriate microtube of the Grifols DG Gel card.
3.3 Add 25 μL of Anti-Fy reagent to the microtube of Grifols DG IgG/Coombs cards.
3.4 Mix gently and incubate at 37°C for 15 minutes.
3.5 Centrifuge card at appropriate time and speed according to card manufacturer’s instructions.
3.6 Read macroscopically after centrifugation. The use of a reader must be validated by the user.

4. BIOVUE COLUMN TECHNIQUE
4.1 Prepare a 3-5% or 0.8% suspension of test red cells in Isotonic saline or PBS or a 0.8% suspension in Ortho 0.8% Red Cell Diluent.
4.2 Add 10 μL of 3-5% or 50 μL of 0.8% suspension of test red cells to the appropriate reaction chamber of the Ortho BioVue cassette.
4.3 Add 40 μL of Anti-Fy reagent to the appropriate reaction chamber.
4.4 Mix gently and incubate at 37°C for 15 minutes.
4.5 Centrifuge the cassette at appropriate time and speed according to cassette manufacturer’s instructions.
4.6 Read macroscopically or with a reader. The use of a reader must be validated by the user.

LIMITATIONS
False positive results are possible when testing a sample with a positive direct antiglobulin test. The inclusion of an auto-control is recommended if this circumstance is suspected.

Do not use with enzyme treated red cells as antigens in the Duffy system may be destroyed by exposure to proteolytic enzymes.

False positive or false negative results may occur through contamination of test materials or any deviation from the recommended techniques.

PERFORMANCE CHARACTERISTICS
Anti-Fy (cell line P3TIM) monoclonal, human IgG blood grouping reagent, NW, has been tested by the indirect antiglobulin technique against donor and clinical specimens collected in anticoagulant. The sample population represented all major phenotypes. The total number of tests (n), and the calculated sensitivity and specificity for each technique are displayed below.

<table>
<thead>
<tr>
<th>TECHNIQUE</th>
<th>Anti-Fy Product Code NW</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>IAT</td>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Bio-Rad Gel</td>
<td></td>
<td>221</td>
<td>100</td>
</tr>
<tr>
<td>Grifols Gel</td>
<td></td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>Column</td>
<td></td>
<td>87</td>
<td>100</td>
</tr>
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Definitions from the Common Technical Specifications (CTS):
Diagnostic Sensitivity: The probability that the device gives a positive result in the presence of the target marker.
Diagnostic Specificity: The probability that the device gives a negative result in the absence of the target marker.

REFERENCES