BLOOD GROUPING REAGENTS

Anti-Fy^{a} (FY1) **REF 210531**

Anti-S (MNS3) **REF 210538**

Anti-s (MNS4) **REF 210539**

(Human/Murine Monoclonal IgG)

For tube technique-By Indirect Antiglobulin Test

- For *In Vitro* Diagnostic Use
- Meets FDA potency requirements
- Discard if turbid
- Preservative: 0.09% (w/v) sodium azide, 0.02% sodium arsenite

INTENDED USE

These reagents are designed to determine the presence of blood group antigens, antigens Fy^{a} (FY1), S (MNS3) and s (MNS4) on the surface of human red blood cells by manual method.

SUMMARY AND EXPLANATION

The Duffy (Fy), S and s̄ antigens are proteins present on the surface of the red blood cell.

The Duffy (Fy) antigen was, first identified in 1950, from antibody in the serum of a multi-transfused hemophilic patient. Duffy antibodies are implicated in mild to severe HTR or HDN.

The s antigen was identified in 1951 and found to be antithetical to S antigen first identified in 1947. They are antigens of the MNS system, and are carried on glycophorin molecules.
They are implicated on no to moderate (rare) HTR and no to severe (rare) HDN.

PRINCIPLE OF THE TEST

The manual technique employed in a tube, utilizes the principle of hemagglutination. Test red blood cells bearing an antigen indirectly cause agglutination in the presence of the corresponding antibodies contained in the reagent (indirect hemagglutination method: antiglobulin test).
The reaction is conducted in two stages. The red blood cells for testing are exposed to the antibody. The antibodies bind to the red blood cells bearing the corresponding antigen. After washing, addition of Anti-Human Globulin Anti-IgG induces agglutination of the sensitized red blood cells bearing the corresponding antigen.

REAGENTS

These reagents contain sodium azide (<0.09%), sodium arsenite (0.02%) and bovine albumin. Any bovine materials used in the manufacture of these products are sourced from donor animals that have been inspected and certified by Veterinary Service inspectors to be disease free.

The reagents are produced by DIAGAST from monoclonal antibodies derived from the *in vitro* culture supernatant of human/murine heterohybridomas.

These reagents are provided with calibrated droppers.

Code	Product Designation	Packaging
210531	Anti-Fy^{a} (FY1)	1 x 3 mL
210538	Anti-S (MNS3)	1 x 3 mL

210539 Anti-s (MNS4) 1 x 3 mL

WARNINGS AND PRECAUTIONS

- These reagents contain <0.09% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide build-up. Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.
- Use proper Personal Protective Equipment according to local SOPs or guidelines.
- All materials that have come into contact with the samples are to be handled as potentially infectious products.
- Special protective measures and conditions for disposal and disinfection should be implemented in accordance with local regulations.
- For *In Vitro* Diagnostic Use
- Do not use beyond expiration date.
- Do not use damaged or leaking reagents.
- Do not use if turbid.
- Do not dilute.
- The absence of all viruses has not been determined in these reagents.
- These reagents have components (Dropper bulb) containing dry natural rubber which may cause allergic reactions
- These reagents contain material of human or animal origin and may transmit infectious agents and should be handled with extreme caution.

"CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THESE PRODUCT WERE DERIVED WERE FOUND NEGATIVE WHEN TESTED FOR HIV, HBV AND HCV. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS."

STORAGE AND STABILITY

- Store reagents at 2°C to 8°C when not use. Do not freeze.
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to the specimen collection.
The blood samples collected following standard blood sampling guidelines in EDTA, heparin or sodium citrate anticoagulant should be stored at 2-8°C.
They should be tested within the following validated hold times:
- Clotted specimens or blood drawn into sodium citrate or EDTA should be tested within 7 days.
- Blood drawn into heparin should be tested within 2 days.

Red blood cells from bags collected in ACD, ACD with AS-1, CPD, CPD with AS-1, CPDA-1, CP2D and CP2D with AS-3 can also be used up to 7 days after the expiration date indicated on the label of the bag.
Do not use blood specimens that exhibit contamination.

MATERIALS

Material provided:
- Anti-Fy^{a} (FY1) (REF 210531): Monoclonal antibody. Anti-Fya IgG human/murine clone DG-FYA-02.
- Anti-S (MNS3) (REF 210538): Monoclonal antibody. Anti-S IgG human/murine clone P3S13JS123.
- Anti-s (MNS4) (REF 210539): Monoclonal antibody. Anti-s IgG human/murine clone P3YAN3.

Material required but not provided:
- Test tubes, tube rack.
- Pasteur pipettes (drop volume 40 to 50 µl) or Automatic pipettes with adjustable precision.
- Centrifuge of relative force from 100 to 1200 rcf.
- Incubator or water-bath at 37°C ± 1°C.

- Isotonic saline solution (0.9% NaCl).
- Positive control blood samples of guaranteed phenotype are required expressing the corresponding heterozygous antigen. Similarly, for a negative control, blood samples should be used which lack the antigen corresponding to the reagent used.
- IgG-sensitized red blood.

Other required complementary reagents:
- Anti-Human Globulin Anti-IgG (such as REF 210547/REF 210549)
- LISS (such as REF 210544).

TEST PROCEDURE

1. In a test tube, prepare a 3-5% red blood cell suspension in LISS.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.
4. Shake to mix and incubate at 37°C ± 1°C for 10 minutes.
5. Wash the red blood cells with isotonic saline solution 3 times and discard the remaining liquid from the last wash.
6. Using the vial dropper, add 1 drop of 'Anti-Human Globulin Anti-IgG' to the red blood cell pellet.
7. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
8. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
9. Read and record the reaction immediately. It is recommended grading positive reactions.

RESULTS

Positive result: If agglutination is present (the red blood cells form one or several clump(s)), the reaction is positive and the antigen corresponding to the reagent used is present on the tested red blood cells.

Negative result: If there is no agglutination (the red blood cells reform a homogeneous suspension), the reaction is negative and the antigen is not present on the tested red blood cells.

Interpretation: The reaction can only be interpreted if:
- the analytical system has been validated with control samples of guaranteed phenotype.
- the direct antiglobulin test on the red blood cells is negative.
- a negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (Refer to the Instructions For Use of the corresponding RBC reagent).

QUALITY CONTROLS

The use of samples of guaranteed extended phenotyping as control samples allows the user to detect anomalies with (handling, reagents, apparatus and the environment) and to implement corrective actions as required.
Known sample controls should be run in parallel on each day of use.
- a sample possessing the antigen corresponding to the antibody in the reagent used,
- a sample devoid of the antigen corresponding to the antibody in the reagent used.

If an unexpected control result is obtained, a complete assessment of the reagents and material used should be made.

A negative reaction obtained in an indirect antiglobulin test should be validated with IgG-sensitized red blood cells (refer to the Instructions For Use of the corresponding RBC reagent).

LIMITATIONS OF THE PROCEDURE

- These reagents are not to be used in a method not described in this Instructions For Use.
- The use of complementary reagents other than the ones cited in the section entitled “MATERIAL - Other required complementary reagents” is of the entire responsibility of the user and must be validated.
- It is recommended to use the calibrated dropper provided in the vial to dispense a reagent drop.
- The reactions are to be read immediately after centrifuging and resuspending.

- False positive or false negative can occur due to improper centrifugation.
- It is imperative to work with clean apparatus and uncontaminated products (bacterial or other contamination).
- Strict compliance with the following is required:
  - storage conditions,
  - equipment calibration is recommended.
- No reagent can guarantee the detection of all the antigenic profiles rare, weak or variants.

SPECIFIC PERFORMANCE CHARACTERISTICS

- These reagents meet FDA potency requirements for Blood Grouping Reagents to be used in tube technique.
- Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
- The device performance is guaranteed only if they are used in the proposed technique and reagents to be used in combination mentioned in this Instructions For Use (ex: Anti-Human Globulin Anti-IgG (REF 210547/REF 210549)).
- Use and validation of other reagents used in combination with the devices, other than those indicated in paragraph titled " MATERIAL - Other required complementary reagents", is possible but only on the user's responsibility.
- DIAGAST denies all responsibility in cases where the devices are not used in accordance with this Instructions For Use.
- The performance of the reagents was confirmed against FDA-licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites. The estimated percent agreements and their lower limits of 95% one-side confidence interval for all sites combined are indicated on the table below.

Table 1. Overall Statistical Analysis results of the comparison study

Reagent	Nº of samples	Negative Percent Agreement (Lower 95% CI)	Nº of samples	Positive Percent Agreement (Lower 95% CI)
Anti-Fy[a]	507	99.80% (99.07%)	773	100% (99.61%)
Anti-S	593	99.83% (99.20%)	679	99.85% (99.30%)
Anti-s	132	99.24% (96.46%*)	1140	99.91% (99.58%)

*Lower value for the Negative Percent Agreement lower confidence bound was obtained due to the limited quantity of s negative samples because of the low frequency of the s negative phenotype in the United States population.

Percent of Agreement only indicates agreement between the DIAGAST reagents and the FDA-licensed reagents and does not indicate which reagent gave the correct result(s).

BIBLIOGRAPHY

- Technical Manual. 20th ed. Bethesda, MD: American Association of Blood Banks, 2020.
- Standards for Blood Banks and Transfusion Services. 32nd ed. Bethesda, MD: American Association of Blood Banks, 2020

DATE OF ISSUE
2021-05-10

Manufactured by:
DIAGAST 251, Av. Eugène Avinée, Eurasanté Parc
59120 Loos – France
U.S. License No.: 1744

For Grifols:
Grifols Diagnostic Solutions Inc.

4560 Horton St. - Emeryville, CA 94608 - USA

Technical Service:
Emeryville, CA, USA 94608
Telephone (in U.S.): (800)452-6877
Or: (510)923-3757
Email: service.americas@grifols.com

SYMBOLS KEY

One or more of these symbols may have been used in the labeling/packaging of this product.