

BLOOD GROUPING REAGENTS

Anti-A (ABO1)

REF 210520

Anti-B (ABO2)

REF 210521

Anti-A,B (ABO3)

REF 210522

(Murine Monoclonal IgM)

For tube technique

- For *In Vitro Diagnostic Use*
- Meets FDA potency requirements
- Discard if turbid
- Preservative: <0.1% (w/v) sodium azide

INTENDED USE

These reagents are designed to determine the presence of ABO system blood group antigens, A and/or B, on the surface of human red blood cells by manual method.

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION

The ABO system was the first human blood group system discovered by Landsteiner in 1900 and is still the most important in transfusion practice. The ABO system is defined by the presence or absence of the A and/or B antigens on human red blood cells and by the presence of antibodies in the plasma or serum corresponding to the antigen or antigens missing in the red blood cells.

ABO group determination is defined by both the demonstration of antigens A and/or B on the surface of human red blood cells and by the presence or absence of Anti-A and/or Anti-B antibodies in the plasma. It is therefore appropriate to identify the erythrocytic antigens using known Anti-A, Anti-B and Anti-A,B reagents (red blood cell test), then to confirm the preceding results by verifying the presence of the corresponding antibodies in the plasma from the test blood using known red blood cells A1, B and, possibly, A2 and O (plasma test).

THE PRINCIPLE ANTIGENS AND ANTIBODIES OF ABO SYSTEM:

| ABO Blood Group | Antigen present on the red blood cells | Antibodies regularly present in the serum/plasma |
|------------------------|---|---|
| O | neither A or B | anti-A and anti-B |
| A | A | anti-B |
| B | B | anti-A |
| AB | A and B | none |

PRINCIPLE OF THE TEST

The manual technique employed in a tube, utilizes the principle of hemagglutination. Test red blood cells bearing an antigen agglutinate in the presence of the reagent containing the corresponding antibody in the direct hemagglutination method.

For ABO group determination, it is therefore appropriate to confirm the results by verifying the presence of the corresponding antibodies in the plasma from the test blood using known red blood cells A1, B and, possibly, A2 and O (plasma test).

REAGENTS

These reagents contain sodium azide (<0.1%), sodium arsenite (0.02%) and bovine albumin. All reagents contain bovine materials. 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 murine hybridomas.

These reagents are provided with calibrated droppers.

| Code | Product Designation | Packaging |
|--------|---------------------|-----------|
| 210520 | Anti-A (ABO1) | 5 x 10 ml |
| 210521 | Anti-B (ABO2) | 5 x 10 ml |
| 210522 | Anti-A,B (ABO3) | 5 x 10 ml |

WARNINGS AND PRECAUTIONS

- These reagents contain <0.1% (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.
- Wear gloves and safety spectacles and handle samples of human origin with caution.
- 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. No known test method can offer complete assurance that products derived from human sources 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 as follows:

- 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-A (ABO1) (REF 210520): Monoclonal antibody. Anti-A IgM murine clone 9113D10.
- Anti-B (ABO2) (REF 210521): Monoclonal antibody. Anti-B IgM murine clone 9621A8.
- Anti-A,B (ABO3) (REF 210522): Monoclonal antibodies IgM blend. Anti-A murine clone 9113D10 and Anti-AB murine clone 152D12.

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.
- Timer.
- Isotonic saline solution (0.9% NaCl).
- Positive control blood samples of guaranteed group typing are required carrying the corresponding antigen and similarly for a negative control, blood samples should be used which lack the antigen corresponding to the reagent used.

TEST PROCEDURE**Direct method in a test tube at room temperature**

1. In a test tube, prepare a 3-5% unwashed red blood cell suspension in isotonic saline solution.
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, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
5. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
6. Read and record the reaction immediately. It is recommended grading positive reactions.

RESULTS

Positive Result: If there is agglutination (the red blood cells form one or several clump(s)), the reaction is positive and the antigen or at least one of the antigens 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 ABO group typing.

QUALITY CONTROLS

The use of samples of guaranteed ABO group typing as control samples allows the user to detect anomalies with (handling, reagents, apparatus and the environment) and to implement corrective actions as required.

Known samples control 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.

LIMITATIONS OF THE PROCEDURE

- These reagents are not to be used in a method not described in this Instructions For Use
- 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 test tube technique.

- Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
- The tests conducted on particular red blood cells of weak phenotype ABO showed good specificity vis-à-vis phenotypes A3 and B3.
- Anti-A,B (ABO3) recognizes red blood cells Ax.
- Anti-B (ABO2) does not agglutinate the “acquired B” red blood cells tested.
- In certain cases (transfusion recipients, certain weak phenotypes A or B (A3, B3...), certain hemopathological modifications, mosaics or chimeras, etc.), an image of a double population may be observed.
- Antibody Anti-A and, accessorially, Antibody Anti-A,B yield a cross-reaction with Antigen Tn which gives rise to an image of a double population (exceptional phenomenon).
- 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 | | | | | |
|--|-----|------|--------------|-------------------|---------------------|
| | | N | Lower 95% CI | Percent Agreement | Acceptance Criteria |
| Anti-A | NPA | 1811 | 99.83% | 100% | 99% |
| | PPA | 1222 | 99.61% | 99.92% | 99% |
| Anti-B | NPA | 2544 | 99.88% | 100% | 99% |
| | PPA | 489 | 99.39% | 100% | 99% |
| Anti-A,B | NPA | 1447 | 99.79% | 100% | 99% |
| | PPA | 1586 | 99.60% | 99.87% | 99% |

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. 18th ed. Bethesda, MD: American Association of Blood Banks, 2014.
- Standards for Blood Banks and Transfusion Services. 30th ed. Bethesda, MD: American Association of Blood Banks, 2016.
- Mannessier L. The use of monoclonal Antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion, Rome, June 1992.

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Manufactured by:

DIAGAST 251, Av. Avinée – 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 of more of these symbols may have been used in the labeling/packaging of this product.

| | |
|---|---|
|  | <i>In vitro</i> diagnostic medical device |
|  | Batch code |
|  | Use by YYYY-MM-DD or YYYY-MM |
|  | Temperature limitation |
|  | Consult instructions for use |
|  | Catalog number |
|  | This way up |
|  | Fragile, handle with care |
|  | Keep dry |