NS Bio-TEc Anti-A, Anti-B, Anti A, B

Monoclonal (IgM) Blood Grouping Reagents

REF. ANTI-ABD 0310

ANT-A-0110 (10ml) (10x10ml)
ANT-B-0110 (10ml) (10x10ml)
ANTI-AB-1010 (10ml) (10x10ml)

INTENDED USE

NS Bio-Tec Diagnostics Anti-A , Anti-B and Anti-A,B reagent is intended for the detection of Blood groups A, B and A,B in Human Blood .

BACKGROUND

Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity. Human red Blood cell antigens can be divided into four groups A, B, AB and 0 depending on the presence or absence of the corresponding antigens on the red blood cells. Approximately 41% of the Caucasian population have the A antigen, 9% have the B antigen, 4% have both A and B antigens, while remaining have neither A nor B antigen

ASSAY PRINCIPLE

Human red blood cells possessing A and/or B antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with Anti-A, Anti-B, Anti-A,B reagents is a positive test result and indicates the presence of the corresponding antigen.
Absence of agglutination of red blood cells with Anti-A, Anti-B, Anti-A,B reagents is a negative test result and indicates the absence of the corresponding antigen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagent should be discarded.
4. ***NS Bio-Tec*** blood grouping reagents are not from human sources, hence contamination due to HB s Ag and HIV is practically excluded.

REAGENTS

NS Bio-Tec Anti-A, Anti-B and Anti-A,B are ready to use reagents prepared from supernatants of mouse hybridoma cell cultures. These antibodies of immunglobulin class lgM are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognising different epitopes of the human red blood cell antigens A and B. Each batch of reagent undergoes quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY

Store the reagent at 2-8 oC. Don't FREEZE.
The shelf life of reagent is as per the expiry date mentioned on the reagent vial label

SPECIMEN COLLECTION AND STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8 ^{o}C if not tested immediately. Do not use haemolysed samples.
Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

EDTA or HEPARIN	: 2 days
Sodium citrate or Sodium oxalate	: 14 days
ACD or CPD	: 28 days

Clotted whole blood should be tested within 14 days.

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (50x75 mm), Test tubes (12x75 mm), Pasteur pipettes, isotonic saline, Centrifuge, timer, mixing sticks.

PROCEDURE

Bring reagent and samples to room temperature before testing.

SLIDE TEST

1. Place one drop of *NS Bio-Tec Anti-A or Anti-B or Anti-A, B* reagent on a clean glass slide.
2. To each reagent drop, add one small drop (50 ul) of whole blood.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm². Rock the slide gently, back and forth.
4. Observe for agglutination macroscopically at two minutes.

TUBE TEST

1. Prepare a 2-3% suspension of the red cells to be tested in Isotonic saline.
2. Place one drop of *NS Bio-Tec Anti-A, Anti-B, Anti-A, B* into correspondingly labeled test tubes.
3. Pipette into each of the test tubes, one drop (50ul) of the test red cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g) or incubate at room temperature for 20-30 minutes.
5. Gently resuspend the cell button, observing for agglutination macroscopically.

INTERPRETATION OF RESULTS

SLIDE AND TUBE TESTS

Agglutination is a positive test result and indicates the presence of A and/or B antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test results and indicates the absence of A and/or B antigen.

REMARKS

1. **(a)** ***NS Bio-Tec*** Anti-A, Anti-B and Anti-A.B reagent do not show a reaction with crypt antigens (T, Tn, Tk activated cells).
   (b) ***NS Bio-Tec*** Anti-B is truly negative reacting with acquired B characteristics.
2. In the tube test procedure, it is recommended that tubes with negative reactions should be recentrifuged and results read again after 5 minutes so that weak antigens are not overlooked.
3. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and determine the time required for achieving the desired results.
4. Results of forward grouping obtained by using ***Anti-A, Anti-B, Anti-A,B*** reagents should always be reconfirmed by performing reverse grouping with known red cells.
5. It is strongly recommended that red cells with known ABC characteristics should be occasionally run, preferably on a daily basis so as to control reagent performance and validate test results.
6. After usage the reagents should be immediately recapped and replaced to 2-8 ºC storage.
7. The label minimum titre claim is based on A_1 group cells for ***NS Bio-Tec Anti-A*** reagent, B group cells for ***NS Bio-Tec Anti-B*** reagent and A_1B cells for ***NS Bio-Tec Anti A,B*** reagent. This is based on titration procedure as recommended by the manufacturer, Any deviation in test procedure could result in variable results.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497.

Lee H.H., Rouger P, Germain C., Muller A. & Salmon C. (1983),
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NS Bio-Tec

NS Bio-Tec Anti-D (Rho) Plus

Monoclonal IgM & IgG Blood Grouping Reagent

REF. ANT-D-0110 (10 ml) (10X10ml)

INTENDED USE

NS Bio-Tec Diagnostics Anti-D (Rho) plus reagent is intended for the detection of Rho (D) Type in human Blood

BACKGROUND

Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or are derived from a human B cell line through EBV transformation. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity. Human red Blood cells are classified as Rho (D) positive or Rho (D) negative depending on the presence or absence of Rho (D) antigen on them. Approximately 85 % of the Caucasian population is Rho (D) positive. The *D^{u}* phenotype is a traditional definition to describe the weak / partial D´s that can be detected with spectrum anti-D Rho (IgM & IgG). About 60 % of the *D^{u}*s (weak / partial D´s) may react with **NS Bio-Tec Anti-D (Rho) plus** reagent in slide test and about 90 % may be detected by the tube technique.

ASSAY PRINCIPLE

Human red blood cells possessing D antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with ***NS Bio-Tec Anti-D (Rho) plus*** reagent is a positive test result and indicates the presence of D (Rho) antigen. No agglutination with ***NS Bio-Tec Anti-D (Rho) plus*** reagent is a negative test result and indicates the absence of the D (Rho) antigen.

All negative test results should be further tested for *D^{u}* (weak / partial D´s) by performing the *D^{u}* test procedure, as described later.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagent should be discarded.
4. ***NS Bio-Tec Anti-D (Rho) plus*** reagent is not from human source, hence contamination due to HB s Ag and HIV is practically excluded.

REAGENTS

NS Bio-Tec Anti-D (Rho) plus is ready to use reagent prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation and is a blend of monoclonal antibodies of immunoglobulin class IgM and IgG . These antibodies are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognising different epitopes of the human red blood cell

antigen D (Rho).

NS Bio-Tec Anti-D (Rho) plus reagent is a blend of IgM and IgG class of Anti-D (Rho) monoclonals, a characteristic which accords versatility to the reagent. It gives an avid saline reacting slide / tube test reagent the capability of detecting *D^{u}* (weak / partial D´s) in the Anti-human globulin phase. Each batch of reagent undergoes quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8 ^{o}C. DO NOT FREEZE.
2. The shelf life of reagent is as per the expiry date mentioned on the reagent vial label

SPECIMEN COLLECTION AND STORAGE

No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8 ^{o}C if not tested immediately. Do not use haemolysed samples. Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

EDTA or HEPARIN	: 2 days
Sodium citrate or Sodium oxalate	: 14 days
ACD or CPD	: 28 days

Clotted whole blood should be tested within 14 days.

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (50x75 mm), Test tubes (12x75 mm), Pasteur pipettes, isotonic saline, Centrifuge, timer, mixing sticks.
Anti Human Globulin (Coombs) reagent

PROCEDURE

Bring reagent and samples to room temperature before testing.

SLIDE TEST

1. Place one drop of **NS Bio-Tec Anti-D (Rho) plus** reagent on a clean glass slide.
2. Add one equal drop of whole blood on the slide.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm^{2}. Rock the slide gently, back and forth.
4. Observe for agglutination macroscopically at two minutes.

TUBE TEST

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of **NS Bio-Tec Anti-D (Rho) plus into the labelled test tubes.**
3. Pipette into each of the test tubes, one drop of the 5 % red cell suspension and mix well,
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button, observing for agglutination macroscopically

D^U TEST PROCEDURE

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of **NS Bio-Tec Anti-D (Rho) plus** into labelled test tube.
3. Add to the test tube, one drop of the 5 % red cell suspension and mix well, incubate at 37 ºC for 15 minutes
4. Wash the contents of the tube at least three times , with isotonic saline and decant completely after the last wash.
5. Add 2 drops of **Anti Human Globulin reagent and** mix well.
6. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
7. Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

SLIDE AND TUBE TESTS

(a) Agglutination is a positive test result and indicates the presence of D (Rho) antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates absence of D (Rho) antigen.

(b) Cord cells heavily sensitised with Anti- D (Rho) may give a false negative immediate spin test rresult

D^U TEST PROCEDURE

(a) Agglutination with the Anti Human Globulin reagent and no agglutination with the control test indicates the presence of the D^u antigen (weak / partial D´s) . No agglutination with both indicates the absence of the D^u antigen (weak / partial D´s)

(b) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^u antigen (weak / partial D´s)

REMARKS

1. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and determine the time required for achieving the desired results.
2. It is strongly recommended that red cells with known Rho (D) positive and Rho(D) negative be occasionally run, preferably on a daily basis so as to control reagent performance and validate test results.
3. After usage the reagents should be immediately recapped and replaced to 2-8° C storage.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497.
2. Lee H.H., Rouger P, Germain C., Muller A. & Salmon C. (1983), The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on Monoclonal antibodies.
3. Human Blood Groups by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995.
4. HMSO, Guidelines for the Blood Transfusion Services, 2nd Ed., 1994.
5. AABB Technical manual, 13th Ed., 1999.

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