Medsource Ozone Biomedicals Pvt. Ltd.

Ozoclone
(Blood Group Sera)
Anti-A, Anti-B, Anti-AB

INTENDED USE:
The monoclonal blood group sera is used to detect the antigens of the ABO blood group system by agglutination of human red blood cells.

PRINCIPLE:
The human ABO blood group system is defined by presence or absence of the antigens A and/or B on the erythrocytes.
The specific monoclonal antibodies of the blood group sera agglutinate red blood cells possessing the homologous antigen or antigens. No agglutination indicates the absence of the relevant antigen.

REAGENT COMPOSITION:
Anti-A, Anti-B and Anti-AB are ready to use blood group sera prepared from supernatants of mouse hybridoma cell cultures. These antibodies of the immunoglobulin class IgM are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognizing different epitopes of the human red blood cell antigens-A and B. Additional stabilizers and preservatives are also added.

SHELF LIFE & STORAGE:
If stored at 2-8°C, the blood group sera can be used until the date indicated on the label. After opening, they should be stored well closed at 2-8°C. While performing the test, the sera can be stored for 2-3 hours at 18-26°C. Avoid bacterial contamination. Do not use if markedly turbid.

SPECIMEN COLLECTION & STORAGE:
Blood specimens are collected on anticoagulants (EDTA, citrate). Freshly drawn specimens are preferred for testing. Specimens exhibiting gross hemolysis should not be used. If not used immediately store specimen at 2-8°C.

PROCEDURE:

1. Slide or plate test

1.1 Place 1 drop (40-50 µl) of blood group sera Anti-A, Anti-B and Anti-AB respectively, on a properly labeled clean plate/slide.
1.2 To each drop of blood group sera, add 1 drop (40-50 µl) of a 5-10% suspension of the red blood cells to be tested (in saline) or whole blood. Mix carefully with a mixing stick.
1.3 Continue rotating or rocking the plate or slide slowly for 2 minutes.
1.4 Observe for agglutination macroscopically.

2. Tube test

2.1 Prepare a 3-5% suspension of the red blood cells to be tested (washed or unwashed) in physiological normal saline.
2.2 Place 1 drop (40-50 µl) of blood group sera Anti-A, Anti-B and Anti-AB respectively, in three small (e. g. 70 × 8 mm), properly labelled test tubes.
2.3 Add 1 drop (40-50 µl) of the red blood cell suspension to each tube and mix thoroughly.
2.4 Centrifuge for 1min at 1000 rpm or incubate at room temperature for 20-30min.
2.5 Gently shake the tube to loosen the sediment from the bottom of the tube.
2.6 Observe for agglutination macroscopically.

Additional Materials required for testing are test tubes (75 × 12 mm or 70 × 8 mm), plates, pipettes (drop volume 40-50 µl), slides, applicator sticks, sodium chloride solution, laboratory centrifuge and timer.

INTERPRETATION OF TEST RESULTS:
Slide & Tube test: 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 result and indicates the absence of A and/or B antigen.
Agglutination results are interpreted follows for Phenotyping

Sample Red cell reacted with				Result
Ozoclone A	Ozoclone B	Ozoclone AB	Saline	
+	-	+	-	A group
-	+	+	-	B group
+	+	+	-	AB group
-	-	-	-	O group

PRECAUTIONS AND LIMITATIONS:

1. In the tube test procedure, it is recommended that tubes with negative reactions should be re centrifuged and results read again after 5 minutes so that weak antigens are not overlooked.
2. If tube tests are not read immediately following centrifugation, dissociation of the reaction may occur leading to a false negative result, especially when cells with weak antigenic expression are tested.
3. It is recommended to run positive and negative controls according to local regulations.
4. Results of forward grouping obtained by using Anti-A, Anti-B, Anti-AB blood group sera should always be reconfirmed by performing reverse grouping with known red cells.
5. The interpretation of reactions obtained when testing infant blood may be complicated by the fact that the infant's serum does not necessarily contain antibody for any antigen absent from the cells.
6. After usage blood group sera should be immediately recapped and replaced to 2-8°C storage.
7. Monoclonal test sera Anti-B does not react with acquired B.
8. The test sera contain sodium azide; avoid any contact with the skin or mucous membranes.
9. False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials and improper centrifugation.
10. When tests are performed on cord blood specimens, passive anti A and/or anti B antibodies from the mother's circulation may yield conflicting results. Cord blood specimens may also give weaker than normal reaction in the cell grouping as the ABH antigens are imperfectly developed at birth.

Warning:
The Manufacturer and Distributors of this product shall not be liable for any losses, liability, claims, costs or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis., whether positive or negative in the use of this product.

Disclaimer:
Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the Manufacture and Distributor and the result may accordingly be affected by environmental factors and user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

Ozoclone
(Blood Group Sera)
Anti-D (IgM) & Anti-D (IgG+IgM)

INTENDED USE:
The monoclonal blood group sera is used to detect the Rh antigen D by agglutination of human red blood cells.

PRINCIPLE:
Human red blood cell possessing D antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with Anti-D (IgM) or Anti-D (Rho)(IgG+ IgM) blood group sera is a positive test result and indicates the presence of the D(Rho) antigen. No agglutination with Anti-D (IgM) or Anti-D (Rho)(IgG+ IgM) sera reagent is a negative test result and indicates absence of the D(Rho) antigen. All negative test results should be further tested for D (weak/ partial D's) by performing the antiglobulin indirect method test procedure, as described later.

REAGENT COMPOSITION:
Anti-D (IgM) and Anti-D (IgG+IgM) are ready to use blood group sera prepared from cell culture supernatant of the heterohybridoma cell line. These antibodies of the immunoglobulin class IgM (For Anti-D IgM) and Class IgG + IgM (For Anti-D IgG + IgM) 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 D (Rho). Anti-D (IgG+IgM) has the additional ability to detect weak/partial D using anti globulin indirect method.

SHELF LIFE & STORAGE:
If stored at 2-8°C, the test sera can be used until the date indicated on the label. After opening, they should be stored well closed at 2-8°C. While performing the test, the sera can be stored for 2-3 hours at 15-30°C. Avoid bacterial contamination. Do not use if markedly turbid.

SPECIMEN COLLECTION & STORAGE:
Blood specimens are collected on anticoagulants (EDTA, citrate). Freshly drawn specimens are preferred for testing. Specimens exhibiting gross hemolysis should not be used. If not used immediately store specimen at 2-8°C.

PROCEDURE:

1. Slide or plate test

1.1 Place 1 drop (40-50µl) of blood group sera Anti-D(IgM) or Anti-D (IgG+IgM) respectively, on a properly labeled clean plate/slide.
1.2 To each drop of blood group sera, add 1 drop (40-50µl) of a 5-10% suspension of the red blood cells to be tested (in saline) or whole blood. Mix carefully with a mixing stick.
1.3 Continue rotating or rocking the plate or slide slowly for 2 min.
1.4 Observe for agglutination macroscopically.

2. Tube test

2.1 Prepare a 3-5% suspension of the red blood cells to be tested (washed or unwashed) in physiological normal saline.
2.2 Place 1 drop (40-50µl) of blood group sera Anti-D (IgM) and D (IgG+IgM) respectively, in three small (e.g. 70 × 8 mm), properly labeled test tubes.
2.3 Add 1 drop (40-50 µl) of the red blood cell suspension to each tube and mix thoroughly.
2.4 Centrifuge for 1min at 1000 rpm or incubate at room temperature for 20-30 min.
2.5 Gently shake the tube to loosen the sediment from the bottom of the tube.
2.6 Observe for agglutination macroscopically.

Additional Materials required for testing are test tubes (75 × 12 mm or 70 × 8 mm), plates, pipettes (drop volume 40-50µl), slides, applicator sticks, sodium chloride solution, anti human globulin (Coomb's) reagent, laboratory centrifuge and timer.

3. Antiglobulin indirect method for weak/partial D

3.1 Prepare a 3-5% suspension of the red cells to be tested in isotonic saline.
3.2 Place one drop of Anti-D (Rho)(IgM+IgG) sera into a labeled test tube.
3.3 Add to the test tube one drop of cell suspension, mix well and incubate at 37°C for 15 minutes.
3.4 Wash the contents of the tube at least two times, with isotonic saline and decant completely after the last wash.
3.5 Add one drops of Anti Human Globulin and mix well.
3.6 Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
3.7 Very gently, resuspend the cell button observing for agglutination macroscopically.

INTERPRETATION OF TEST RESULTS:
Slide & Tube test: Agglutination is a positive test result and indicates the presence of Anti D (Rho) on red blood cells. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates the absence of Anti D (Rh)antigen. However, this requires to be confirmed by indirect Coomb's test to rule out the possibility of the presence of D antigen, using Anti-D (IgG) and Anti-D (IgG+IgM) monoclonal.

PRECAUTIONS & LIMITATIONS:

1. As under centrifugation or over centrifugation 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. After usage the reagents should be immediately recapped and replaced to 2-8°C storage.
3. The label minimum titre claim is based on Rho (D) positive red cells (phenotype: R r) for Anti-D Monoclonal (IgM+IgG) blood group sera. This is based on titration procedure as recommended by the manufacturer. Any deviation in test procedure could result in variable results.
4. The use of biotechnological production means that the risk of contamination of the test sera with infectious agents is virtually excluded. Since the test sera contain materials of animal origin, they should be treated as a potential health hazard and be handled accordingly.
5. The test sera contain sodium azide; avoid any contact with the skin or mucous membranes.
6. Anti-D (IgM+IgG) can be used for indirect anti globulin test.
7. Slide and microplate techniques are less sensitive in detecting weak/partial D. Therefore, the tube test is recommended for the detection of weak/partial D and variant D antigens.
8. Weaker reactions may be observed with stored blood. Stronger results will be obtained with fresh cells.
9. The donors of the cell lines used to produce this reagent have been tested and found to be non-reactive for HIV and HCV antibodies, HBsAg , EBV and MAP (Mouse Antibody Production) viruses. However, it is recommended to treat the reagent as potentially infectious.
10. Prior to disposal of used reagents appropriate means for inactivation should be applied (e.g. autoclaving or chemical treatment).

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 Blood Transfusion Services, 2nd Ed., 1994.

Symbol	Explanation	Symbol	Explanation
	Manufactured by	IVD	In vitro diagnostic medical device
	No. of Tests		See instruction for use
LOT	Lot No.	2°C 8°C	Temperature Limitation
	Manufacturing Date	REF	Catalogue Number
	Expiry Date		Do not reuse

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