MaxCLONE (ANTI-A, B, D (IgM)

Monoclonal Blood Grouping Antibodies for Slide and Tube Tests

ORDERING INFORMATION

Ref. No.	Pack Size	Presentation
AVABD2-15	3 x 5 ml	Single Liquid Reagent
AVABD2-30	3 x 10 ml	

INTENDED USE :

MaxCLONE ANTI-A, B, D (IgM) reagent kit is intended for use of *in-vitro* determination of the presence or absence of A, B and D (Rho) antigen on human red blood cells by the slide and tube test method.

INTRODUCTION

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, A,B and O 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 the remaining have neither the A nor the B antigen.

PRINCIPLE

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

REAGENTS

MaxCLONE Anti-A, B, D (IgM) are ready to use reagents prepared from the supernatants of mouse hybridoma cell cultures. These antibodies of immunoglobulin class IgM are mixture of several monoclonal antibodies of the same specificity but having the capacity of recognizing different epitopes of the human red blood cell antigens A, B and D (Rho) respectively. Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagent at 2-8^0**C .** DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

KIT CONTAINS

Name of Reagent	Antibody Type	Pack Size
Anti A	IgM	1 x 10, 1 x 5 ml
Anti B	IgM	1 x 10, 1 x 5 ml
Anti D	IgM	1 x 10, 1 x 5 ml

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.
When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Glass slides, Test tubes, Pasteur pipettes, Isotonic saline (0.9% NaCl Solution), Centrifuge, Timer, Mixing sticks, Sodium Hypochlorite (1%).

PRECAUTIONS AND WARNINGS

1. Test for In-vitro diagnostic use only and should be run by competent and trained person only.
2. Not for medicinal use.
3. Use clean and dry glass wares.
4. The reagent contains 0.1% sodium azide as a preservative. Avoid contact with skin & mucosa.
5. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.
6. Always wear hand gloves while performing the test. Avoid re-using gloves or use of washed gloves.
7. Do not smoke, eat and drink in the testing area.
8. Do not use haemolysed specimen for testing.
9. Do not use the reagent beyond expiry date.
10. Do not pipette by mouth.
11. All the materials used in the assay and samples should be decontaminated in 1% sodium hypochlorite. They should be disposed of in accordance with established safely procedure.
12. Spills should be decontaminated promptly with sodium hypochlorite or any other suitable disinfectant.
13. Wash hands thoroughly with soap or any suitable detergent, after the use of kit. Consult a physician immediately in case of accident or contact with eyes.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8^{0}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

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.

Slide Test

1. Place one drop or 50µl of **MaxCLONE** Anti-A or Anti-B or ANTI-D (IgM) reagents using the reagent dropper separately on a clean glass slide.
2. To each reagent drop, **add one small drop (25 µl) of whole blood**.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm^2.
4. Rock the slide gently, back and forth.
5. 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 or 50µl of **MaxCLONE** Anti-A, Anti-B, or ANTI-D (IgM) reagents using the reagent dropper into corresponding labeled test tubes.
3. Pipette into each of test tubes, one drop or 50µl red cell suspension and mix well.
4. Centrifuge for one minute at 1000 RPM (125 g) or 20 sec. 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 result and indicates the absence of A and/or B antigen.

D^u Test Interpation

(a) Agglutination with reagent indicates the presence of D^u antigen (weak / partial D's).
(b) No agglutination with reagent indicates the absence of D^u antigen. Negative reactions obtained in D^u test must be validated:- add 50µl of coomb's control cells to the reaction mixture. A positive reaction confirms the activity of the coomb's reagent and validates the negative reaction before the addition of the coomb's control cells.
(c) Mixed field agglutination in the D^u test on red cells from a recently delivered woman may indicate a mixture of maternal Rho (D) negative and fetal Rho (D) positive blood.
(d) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^u antigen (weak / partial D's).

Slide and Tube Tests

a) Agglutination with the Anti-D (IgM) is a positive test result and indicates the presence of D antigen. Do not interpret peripheral drying or fibrin strands as agglutination.

b) No agglutination with Anti-D (IgM) is a negative test result and indicates the absence of D antigen.

Grades	Description
4+	1 big clump
3+	2 or 3 clumps
2+	many small clumps with clear supernatant
1+	many small clumps with turbid supernatant
W	granular suspension
Zero or -ve	smooth suspension
H	partial or complete hemolysis (positive reaction)

REMARKS

1. (a) **MaxCLONE** Anti-A, Anti-B and ANTI-D (IgM) reagents do not show a reaction with crypt antigens (T, Tn, Tk activated cells).
   (b) **MaxCLONE** 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 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.
4. Results of forward grouping obtained by using **MaxCLONE** Anti-A, Anti-B and ANTI-D (IgM) reagents should always be reconfirmed by performing reverse grouping with known red cells.
5. It is strongly recommended that red cells with known ABO characteristics should be occasionally run, preferably on a daily basis so as to control reagent performance and validate the test results.
6. After usage the reagents should be immediately recapped and replaced to 2-8^{0}C storage.
7. The label minimum titre claim is based on, A group cells for **MaxCLONE** Anti-A reagent, B group cells for Anti-B reagent, AB group cells for ANTI-D (IgM) reagents. This is based on titration procedure as recommended by the manufacturer. Any deviation in test procedure could result in variable results.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagents have been characterized by all the procedures mentioned in the Recommended Techniques.
2. Prior to release, each lot tested by the Recommended Techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen- negative cells.
4. The potency of the reagents has been tested against the minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC).
5. The Quality Control of the reagents was performed using red cells that had been washed at least twice with PBS or Isotonic saline prior to use

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 reagents should be discarded.
4. Reagents are not from human source, hence contamination due to HBsAg, HIV and HCV is practically excluded.

DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those mentioned in the Recommended Techniques.
2. Any deviations from the Recommended Techniques should be validated prior to use.

WARRANTY

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

REFERENCE

1. Human Blood Groups, by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995. ANTI-A, ANTI-B,
2. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity., Nature, 256, 495-497.
3. Lee H.H., Rouger P., Germain C., Muller A & Salmon C. (1983). The pro duction and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on Monoclonal antibodies.
4. Human Blood Groups, by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995. (4) HMSO, Guidelines for Blood Transfusion Services., 2nd Ed., 1994. (5) Blood transfusion in clinical medicine, P. L. Mollison, C. P. Engelfreit, Marcela Conteras, 10th Ed., 1997, Blackwell Scientific Publications.

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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