

# MaxCLONE Anti-D (IgM + IgG)

## Blood Grouping Antibodies for Slide and Tube Tests

### SUMMARY

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 and Rho (D) negative depending upon the presence or absence of D (Rho) antigen on them. Approximately 85% of the Caucasian population are Rho (D) positive. The D<sup>u</sup> phenotype is a traditional definition to describe the weak / partial D's that can be detected with Anti-D (Rho) (IgM+IgG). About 60% of the D<sup>u</sup> (weak / partial D's) may react with Anti-D (Rho) (IgM+IgG) in slide tests and about 90% may be detected by the tube technique.

### REAGENT

**MaxCLONE** Anti-D (IgM + IgG) is a 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 the immunoglobulin class IgM and IgG. These antibodies are monoclonal antibodies of the same specificity but having the capability of recognising different epitopes of the human red blood cell antigen D. **MaxCLONE** Anti-D (IgM + IgG) is a blend of IgM and IgG class of Anti-D monoclonal, a characteristic which accords versatility to the reagent. It gives an avid saline reacting slide / tube test reagent the capability of detecting D<sup>u</sup> (weak/partial D's) in the Anti Human Globulin Phase. 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°C. DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

### 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 **MaxCLONE** Anti-D (IgM + IgG) reagent is a positive test result and indicates the presence of the D antigen. No agglutination with **MaxCLONE** Anti-D (IgM + IgG) reagent is a negative test result and indicates absence of D antigen. All negative test results should be further tested for D<sup>u</sup> (weak / partial D's) by performing the D<sup>u</sup> test procedure, as described later.

### NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. To be used by a qualified personnel. 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.
5. It is necessary to use calibrated dropper provided in the reagent vial to dispense a reagent drop.

6. It is advisable to wear gloves and safety spectacles and handle test specimens of human origin with caution.
7. Do not use damaged or leaking reagents.
8. Special protective measures, conditions for disposal and disinfection should be implemented in accordance with local regulations.

### SAMPLE 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°C if not tested immediately. Do not use haemolysed samples. Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:

4 EDTA or Heparin : 2 days      4 Sodium citrate or sodium oxalate : 14 days  
4 ACD or CPD : 28 days

### ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

Glass slides (60 x 85 mm), Test tubes (12 x 75 mm), Test tube rack, Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks, Anti Human Globulin (Coombs) reagent.

### TEST PROCEDURE

Bring reagents and samples to room temperature before testing.

#### Slide Test

1. Place one drop of **MaxCLONE** Anti-D (IgM + IgG) reagent using the reagent dropper on a clean glass slide.
2. To the reagent drop add a drop of whole blood (Approximately 50µl)
3. Mix well the reagent and blood sample with a mixing stick uniformly over an area of approximately 2.5 cm<sup>2</sup>.
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at the end of two minutes.

#### Tube Test

1. Prepare a 5% cell suspension of the red cells to be tested in isotonic saline.
2. Place one drop of **MaxCLONE** Anti-D (IgM + IgG) reagent into a labeled test tube.
3. Pipette 50µl of test red cell suspension in to the test tube and mix well.
4. Centrifuge for one 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<sup>u</sup> TEST PROCEDURE

1. **Prepare a 5% suspension of the red cells to be tested in isotonic saline.**
2. **Place one drop of MaxCLONE** Anti-D (IgM + IgG) reagent into a labeled test tube.
3. Add to the test tube 50µl of cell suspension under test, mix well and incubate at 37°C for 15 minutes.
4. Wash the contents of the tube thoroughly, atleast three times, with isotonic saline and decant completely after the last wash.
5. Add 100µl of **MaxCLONE Anti Human Globulin** reagent and mix well.
6. Centrifuge for 1 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.



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## INTERPRETATION OF RESULTS

### Slide and Tube Tests

- Agglutination with the **MaxCLONE** Anti-D (IgM + IgG) is a positive test result and indicates the presence of D antigen. Do not interpret peripheral drying or fibrin strands as agglutination.
- No agglutination with **MaxCLONE** Anti-D (IgM + IgG) is a negative test result and indicates the absence of D antigen.

### D<sup>u</sup> Test Procedure

- Agglutination with reagent indicates the presence of D<sup>u</sup> antigen** (weak / partial D's).
- No agglutination with reagent indicates the absence of D<sup>u</sup> antigen. Negative reactions obtained in D<sup>u</sup> 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.
- Mixed field agglutination in the D<sup>u</sup> test on red cells from a recently delivered woman may indicate a mixture of maternal Rho (D) negative and fetal Rho (D) positive blood.
- Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D<sup>u</sup> antigen (weak / partial D's).

## REMARKS

- As undercentrifugation and overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required of achieving the results.
- It is strongly recommended that as a routine quality control measure with known D positive and D negative red cells be occasionally run, preferably on a daily basis to validate reagent performance.
- After usage, the reagents should be immediately recapped and replaced to 2-8°C storage.
- Cord Cells heavily sensitized with Anti-D (Rho) may give false negative result in immediate spin test.
- False positive reactions may occur if the test subject has cold agglutinins.
- MaxCLONE** Anti-D (IgM + IgG) have the feature of recognizing certain rare antigen motif of type (RoHar) and may thus yield discordant results with polyclonal reagents that may or may not recognize them.
- MaxCLONE** Anti-D (IgM + IgG) enables the screening for weak Rh red blood cells in the D<sup>u</sup> test procedure with coomb's reagent.
- The tests conducted on particular phenotypes, while satisfactory, cannot ensure recognition of all weak or variant subjects, due to variability of antigen motifs.

## PERFORMANCE CHARACTERISTICS

The performance of **MaxCLONE** Anti-D (IgM + IgG) comply with the common technical specifications of in-vitro diagnostic medical devices under the recommended methods. The performance of **MaxCLONE** Anti-D (IgM + IgG) was evaluated on over 3035 samples (from donors, patients and neonates) drawn on the recommended anticoagulants. The evaluation demonstrated 100% specificity of reagent versus the expected results with common known Rhesus phenotypes.

## 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.

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Transforming Research into Innovations

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