MeriSera Anti-D (IgG+IgM) Monoclonal
Blood Grouping Reagent

Product Code : DGMSER-01

INTENDED USE

MeriSera Anti-D (Monoclonal-IgG+IgM) is intended to use as a reagent for the detection of the 'D' antigen present on human red blood cell.

INTRODUCTION

As reactive components MeriSera Anti-D (IgG+IgM) blend contains human monoclonal antibodies of the immunoglobulin classes IgG and IgM and is therefore suited for an indirect antiglobulin test. The antibodies are derived from cell culture supernatant and demonstrate the consistent specificity and reproducibility characteristic for monoclonal antibodies. Antibodies are diluted in a buffered protein solution containing bovine albumin.

PRINCIPLE OF THE TEST

Human red blood cells possessing D antigen will agglutinate when mixed with Anti-D antibody, directed towards D antigen.

The Anti-D IgM component of the reagent produces direct agglutination of the red blood cells that is carriers of the normal D antigen. In the most cases weak D does not directly agglutinate with this reagent. The Anti-D IgG component may detect the weak variables by the indirect test with anti-globulin.

Agglutination of red blood cells with Anti-D is a positive test reaction and indicates the presence of D antigens on the RBCs. Absence of agglutination of red blood cells with Anti-D is a negative test result and it indicates the absence of D antigen on the RBCs.

Kit contain following Reagents

No	Reference No	Name of Product	Antibody type	Pack Size
1	DGMSER-01	MeriSera Anti-D	(IgG+IgM)	1x10ml

No	Reference No.	Name of Product	Antibody Type	Colour	Dye Used
1	DGMSER-01	MeriSera Anti-D	(IgG+IgM)	Colourless	None

MATERIALS REQUIRED

Glass slides, test tubes, Pasteur pipettes, isotonic saline (0.9% NaCl solution), centrifuge, timer, applicator sticks, Sodium hypochlorite (1%)

STORAGE OF TEST KIT

Unopened test kits should be stored at **2-8°C** upon receipt. Sodium azide is added to the antibodies at 0.1% concentration as preservative. The test kit may be used till the time of the expiry date mentioned. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. **Do not freeze.**

SAMPLE COLLECTION AND STORAGE

Do not use haemolysed samples. Samples should be collected with a suitable anticoagulant in a sterile container and should be tested immediately. If testing is delayed, blood should be stored at 2-8°C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. Clotted samples should be used within 24 hours of collection.

PRECAUTIONS AND WARNINGS

1. Test for In-vitro diagnostic use only and should be run by competent and trained person only.
2. The reagent contains 0.1% sodium azide as a preservative, Avoid contact with eye, skin & mucosa.
3. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.
4. Always wear hand gloves while performing the test.
5. Do not use the reagent beyond expiry date.
6. Do not pipette by mouth.
7. All the materials used in the assay and samples should be decontaminated in 1% sodium hypochlorite. They should be disposed off in accordance with established safety procedure.
8. Spills should be decontaminated promptly with sodium hypochlorite or any other suitable disinfectant.
9. 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.

CONTROLS AND ADVICE

1. It is recommended a positive control and a negative control to be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. In the Recommended Techniques one volume is approximately 50µl when using the vial dropper provided.
3. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the reagents are in use.
4. The user must determine the suitability of the reagents for use in other techniques.

TEST PROCEDURE

Bring reagent and blood specimen at room temperature before testing.

Test should be performed at room temperature by:

1. **Slide method:-**

1) Add one drop(50µl) of Anti-D (IgG+IgM) on a clean and labeled glass slide.
2) Next to it, add one drop(50µl) of the red blood cell to be tested.
The antiserum cells ratio should be maintained for all the assays.
3) Mix the antiserum and the red blood cells with a clean applicator uniformly.
4) Macroscopically observe the presence or absence of agglutination.
5) If the observation period is longer then the effects of the reagent evaporation may produce erroneous results (weakly positive).
6) Some weak or partial D samples may show absence of agglutination when the slide technique is used. In the presence of indeterminate or negative results, confirm by using the tube technique.

The slide technique is not recommended for weak or partial D samples.

2. **Tube method :-**

1) Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2) Place one drop (50µl) of Anti-D (IgG+IgM) on a labeled tube.
3) Add one drop (50µl)of the red blood cell suspension to be tested.
4) Mix well and incubate at room temperature for 5 minutes.
5) Gently shake tube to mix the contents thoroughly.
6) Centrifuge for 1 minute at 1000 rpm.
7) Gently re-suspend the cell button, observing for agglutination macroscopically.
8) For specificity observe all the negative tubes under the microscope for clear negative reaction.

INTERPRETATION OF RESULTS : (SLIDE TEST AND TUBE TEST)

-- Agglutination is a positive test result and indicates the presence of D antigen.
-- No agglutination is a negative test result and indicates the absence of D antigen.

3. TEST PROCEDURE FOR WEAK D :-

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop (50µl) of MeriSera Anti-D (IgG + IgM) reagent into a labeled test tube.
3. Add one drop (50µ)l of 5% cell suspension into test tube, mix well and incubate at 37°C for 15 minutes.
4. Wash the contents of the tube thoroughly three times with isotonic saline and decant completely after the last wash.
5. Add 100µl of Anti Human Globulin and mix it well.
6. Centrifuge for 1 minute at 1000 RPM .
7. Gently resuspend the cell button and observe for agglutination macroscopically.
8.For specificity observe all the negative tubes under the microscope for clear negative reaction.

INTERPRETATION OF RESULTS :

-- Agglutination with reagent indicates the presence of D antigen (weak / partial D's).
-- No agglutination with reagent indicates the absence of D antigen.
-- Agglutination with reagent and no agglutination with the control indicates the presence of weak D antigen (weak / partial D's).
-- No agglutination with reagent and control indicates the absence of weak D antigen.
-- Mixed field agglutination in the weak D 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 weak D antigen (weak / partial D's).

TROUBLE SHOOTING :

FALSE POSITIVE	
Cause : - Contaminated blood specimen or reagents - Drying in slide test - Clotting of blood	Remedy : -Make sure that there is no contamination of blood specimen or reagent . - Do not read the result after 2 minutes. - Test the sample immediately if anti coagulant is not added to the sample.

FALSE NEGATIVE	
Cause : -Contamination of blood specimen or reagent.	Remedy : - Make sure that there is no contamination of blood specimen or reagent. - Use clean slide and tube for testing. - Do not read the result after 2 minutes.

WEAK/DELAYED REACTION	
Cause : -Prolonged storage of red blood cells. - Expired Reagent	Remedy : - Store the blood sample with anti coagulant at 2-8°C for less then 30 days. -- Check the expiry date on the reagent bottle.

SPECIFIC PERFORMANCE CHARACTERISTICS

1.The reagents have been characterized by all the procedures mentioned in the Recommended Techniques.
2.Prior to release, each lot is 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.

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.

PERFORMANCE LIMITATIONS

1. Slide techniques are not recommended for the detection of weak D or partial RhD samples. All negative slide tests should be confirmed by tube testing to confirm absence of weak subgroups.
2. Certain tests performed on unwashed samples (eg cord), direct antiglobulin test positive samples, or samples stored and tested at 18°C or below, may exhibit false positive reactions due to the potentiators used in the formulation of this reagent. A satisfactory reagent control may be achieved by substituting 6-10% BSA in saline for the blood grouping reagent in the procedure chosen for use. If the control test gives a positive reaction, a valid interpretation of the results obtained in red blood cell testing cannot be made. A control test should always be used if a sample groups as AB RhD positive.
3. Driblocks and waterbaths promote better heat transfer and are recommended for 37°C tests, particularly where the incubation period is 30 minutes or less.
4. Some very weak D and/or partial RhD samples may not react with monoclonal anti-D reagents.
5. The expression of certain red blood cell antigens may diminish in strength during storage, particularly in EDTA and clotted samples. Better results will be obtained with fresh samples.
6. Gently resuspend tube tests before reading. Excessive agitation may disrupt weak agglutination and produce false negative results.
7. Excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.
8. False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

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.

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