

+4- One big clump in the centre.

Titre: Titre is checked by tube method with 2-5% cell suspension. It is defined as the reciprocal of highest dilution of the antibodies which gives agglutination. The reagent is diluted up to 512 dilution by two fold serial dilution in tubes.

Specificity: It is checked to ensure whether the reagent is specific for the purpose or not with Rh negative cells (O⁻).

Haemolysis: It is checked to ensure that cells do not get haemolysed during specificity testing.

Prozone: It is checked by tube method to ensure that no negative reactions are obtained with low dilution of the Antisera.

Rouleaux: It is checked to ensure that the Antisera does not give pseudo agglutination in which the red blood cells look like pile of coins.

Avidity, Intensity, titre, specificity, Haemolysis, prozone and rouleaux of Anti-D (IgM) Monoclonal Antibodies are as per I.P standards.

LIMITATION

The negative reactions are to be completed with a search of low grade D^u antigen using the Indirect Coomb's technique.

PROBLEMS IN Rh TYPING

1. Improper identification of specimen.
2. Improper techniques like
 - Cell to reagent ratio.
 - Failure to identify haemolysis
 - Improper storage of Reagents.
 - Fibrin clots
 - Over incubation of cells and reagents.
3. Improper centrifuge calibration resulting in over/under centrifugation.
4. Problems in Donor/Patients.
 - Weak expression of D antigens.
 - Immunoglobulin coating of red blood cells.
 - Increased abnormal proteins in patients (multiple myeloma) resulting in rouleaux and thus giving false positive results.
- Poly agglutination.

BIBLIOGRAPHY

- KHOLER G., MILSTEIN C., 1975 Continuous culture of fused cells secreting antibody of predefined specificity. Nature, 256, 495 -497

- Blood Group Serology, Boorman, Dodd & Lincoln, Churchill Livingstone, 6th Edition
- Practical Blood Transfusion, Douglas W. Huestis, Bove & Coso, 4th Edition 1988, Little Brown and Co.
- Blood Transfusion in Clinical Medicine, P.L. Mollison, C. T. Engelbreit, Marcela Contreras, 9th Edition, 1993.
- Basic Blood Group Serology, Makroo R.N. 1st Edition 1994.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, in as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application thereof.

in vitro diagnostic Reagent, not for medicinal use

J. Mitra & Co. Pvt. Ltd.

A-180-181, Okhla Ind. Area, Phase - 1, New Delhi - 110 020, India

Phone: +91-11-47130300, 47130500

E-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

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J. Mitra & Co. Pvt. Ltd.

Anti-D (IgM) Monoclonal Antisera/ Antibodies for Rh Typing

The Anti-D (IgM) Monoclonal Antibodies are *in vitro* culture supernatant of hybrids obtained by cellular fusion (Hybridoma technology). The Anti-D (IgM) Monoclonal Antibodies has the following features:

- It agglutinates in saline solution.
- Active at room temperature.
- Usable on glass slide as well as in tube.

PRINCIPLE

Human red cells are classified as Rh positive (Rh⁺) or Rh negative (Rh⁻) depending upon the presence or absence of "D" antigen on them. Major percentage of the population is Rh positive. Human red blood cells possessing D antigen will agglutinate in the presence of corresponding antibody. Agglutination of red cells with Anti-D (IgM) Monoclonal Antibodies indicates the presence of D-antigen and hence Rh positive result. It can detect D antigen (Rh₁) & high grade D^u antigen (weaker variant of Antigen D). In case no agglutination is obtained with Anti-D (IgM) Monoclonal Antibodies, the cells should be then checked for the presence of low grade D^u antigen by Coomb's Test using Anti-D (IgG) & Anti Human Globulin serum (Coomb's Reagent).

STORAGE AND PACK SIZE:

The Anti-D (IgM) Monoclonal Antibodies are packed into 10 ml dropper vials.















The antibodies are stable at 2-8°C until the expiry date mentioned on the reagent vial label. Sodium azide is added to the antibodies at 0.1 % concentration as preservative.

SAMPLE COLLECTION:

Blood sample should be collected with a suitable anticoagulant in a sterile stoppered container & should be tested immediately. If testing is delayed, blood should be stored at 2-8°C & must be examined not later than 48 hours. Haemolysed samples should not be used for testing and clotted blood should be used within 24 hours of collection.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

	Manufactured By		<i>In vitro</i> diagnostic medical device
	Catalogue Number		See Instruction for use
	Lot Number Batch Number		Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date		Keep away from sunlight
	Do not use if package is damaged		Contains biological Material of Animal Origin
	Keep Dry		Country of Manufacture

WARNING FOR USERS



CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
6. Perform the test at room temperature (20-30°C).
7. Do not pipette by mouth.
8. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials

or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.

9. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
10. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
11. The monoclonal antibodies contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)

ADDITIONAL MATERIALS REQUIRED

Glass slides, Test tubes (10X75mm), Pasteur Pipettes, Normal saline, Beakers, Centrifuge, Timer & Mixing sticks.

GROUPING TECHNIQUE

Separate the RBCs from the serum or plasma by centrifuging blood at 5000 rpm for 5 mins.

The Rh Typing is performed at room temperature by:

- (a) Slide or tile method.
- (b) Tube method.
- (c) Microplate Method.

(a) **Slide Method:** It is used mainly for emergency Rh typing especially in out door camps with whole blood sample. Place one drop each of Anti-D (IgM) Monoclonal Antibodies and negative control on a slide and add one drop of whole blood sample to both of them. Mix the cells & antibodies with clean mixing stick & spread the mixture over an area of 2 cm. Rock the slide gently from side to side & observe for the agglutination within one minute. Complete the test by a second observation after two minutes.

(b) **Tube Method (Immediate centrifugation) :** Prepare a 3-4% suspension of red cells washed in isotonic saline solution. Put respectively one drop of

Anti-D (IgM) Monoclonal Antibodies reagent and one drop of 3-4% cells suspension in the tube. Shake to homogenise antibodies and red cells suspension, then centrifuge for one minute at 1000 rpm. The reaction is read macroscopically by shaking gently the tube so as to loosen the cells pellet. If the red cells separate in one or more clumps, the reaction is positive. If the red cells return to a homogeneous suspension, the reaction is negative.

(c) **Microplate Method :** Microplate method is ideal for testing large no. of blood samples. Microplates are polystyrene plates having 96 small wells (either 'v' shaped type, flat bottom or 'U' type). Add one drop each of Anti-D (IgM) MonoClonal Antibodies & negative control to two different wells & add one drop of 2-4% cell suspension to both the wells. Gently shake to mix the antibodies and cells. Incubate at room temperature for 30 mins. Gently shake the plate either by tapping the side of the plate or on microplate shaker. Results are read as in tube method.

INTERPRETATION

SLIDE AND TUBE METHOD

Agglutination of the red blood cells with the antibodies is a positive test indicating cells are Rh positive & absence of agglutination indicates that the cells are either Rh negative or D^u positive.

QUALITY CONTROL

Each batch of Anti-D (IgM) Monoclonal Antibodies is subjected to stringent internal quality control regarding specificity, avidity, intensity and titre to ensure constant quality of antibodies.

Avidity: It is defined as the reactivity time taken by the antibodies to show the agglutination in seconds and is tested by slide method with 40% red cells suspension.

Intensity: It shows the strength of the reaction ie clumping and is tested by slide method with 40% red cells suspension. It is graded as :

- +/- Doubtful for agglutination. Repeat test.
- +1- Small clumps scattered in the test area
- +2- Two or more clumps of equal size
- +3- One big clump with some small clumps