

**ANTI-C, ANTI-c, ANTI-E, ANTI-e**  
**(IgM)**  
MONOCLONAL Rh / hr TYPING ANTIBODIES FOR SLIDE AND TUBE TESTS

**SUMMARY**

The Rh blood group system consists of forty-five antigens, which are expressed on human red blood cells. The Rh antigens are encoded by two highly homologous, closely linked genes. The RHD producing the D antigen and the RHCE producing the Cc and Ee antigens. C and c, E and e, represent two opposing pairs of antigens.

Anti-C, Anti-c, Anti-E and Anti-e reagents are useful:

- For determination of the probable Rh genotype of an individual
- For the selection of donors who have become immunized with the C, c, E or e antigen during pregnancy or transfusion.
- In pre-transfusion testing and prediction of hemolytic disease of the newborn.
- When used in conjunction help to determine the zygosity of the red blood cells tested.

Approximately 70% of the Caucasian population have the C antigen, 30% have the E antigen, 80% have the c antigen and 90% have the e antigen.

**REAGENTS**

Anti-C, Anti-c, Anti-E and Anti-e are ready to use reagents prepared from cell culture supernatant of respective human cell lines.

Each batch of reagents undergoes rigorous quality control at various stages of its manufacture for its specificity, avidity, titre and performance.

**PRINCIPLE**

Human red blood cells possessing the C, c, E, and e antigens will agglutinate in the presence of the antibody directed towards the antigen.

Agglutination of red blood cells with Anti-C, Anti-c, Anti-E and Anti-e reagents is a positive test result and indicates the presence of the corresponding antigen.

Absence of agglutination of red blood cells with Anti-C, Anti-c, Anti-E and Anti-e is a negative test result and indicates the absence of the corresponding antigen.

**NOTE**

The reagent contains sodium azide 0.1 % as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.

Store the reagent at 2-8°C. **DO NOT FREEZE.**

Extreme turbidity may indicate microbial contamination or denaturation of the protein due to thermal damage. Such reagents should be discarded.

The cell lines used to produce ® reagents are from a human source, which has been tested and found to be negative for HIV, HCV and HBsAg. The risk of contamination can be excluded by the use of biotechnology production methods. However, all test reagents should be treated as a potential health hazard and handled accordingly.

The shelf life of the reagents is as per the expiry date mentioned on the reagent vial labels.

**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 hemolysed 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

#### **ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS**

Glass slides (50 x 75 mm), Test tubes (10 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks.

#### **TEST PROCEDURE**

Bring all reagents and samples to room temperature before testing.

##### **Slide test**

1. Place one drop of <sup>®</sup> reagent Anti-C or Anti-c or Anti-E or Anti-e on a clean glass slide.
2. To each reagent drop, add one drop of whole blood.
3. Mix well with a mixing stick uniformly over an area of 2.5 cm<sup>2</sup>.
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 of <sup>®</sup> Anti-C, Anti-c, Anti-E and Anti-e into correspondingly labelled test tubes.
3. Pipette into each of the test tubes, one drop of the test red cell suspension and mix well.
4. Incubate at 37°C for 5 minutes.
5. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
6. Gently resuspend the cell button observing for agglutination.

#### **INTERPRETATION OF TEST RESULT**

##### **Slide and tube test**

Agglutination is a positive test result and indicates the presence of C or c or E and / or e antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative test result and indicates the absence of C or c or E and / or e antigen.

#### **REMARKS**

1. Red cells that have a positive direct antiglobulin test may produce a false positive result. Use Rh / hr control to validate the results.
2. In the tube procedure, it is recommended that tubes with negative reactions should be centrifuged and results read again after 5 minutes so that weak antigens are not overlooked.
3. As undercentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and determine the time required for achieving the desired result.
4. A positive control (ideally heterozygous red blood cells) known to possess C or c or E or e antigens and a negative control (known red blood cells lacking the respective antigen) should be tested in parallel with each batch of tests.

#### **WARRANTY**

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

## **BIBLIOGRAPHY**

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