

Blood Grouping Reagent

Anti-C (RH2)

Seraclone® Human Monoclonal (MS24)

Anti- \bar{c} (RH4)

Seraclone® Human Monoclonal (MS33)

Anti-E (RH3)

Seraclone® Human Monoclonal (MS260/MS12)

Anti-e (RH5)

Seraclone® Human Monoclonal (MS16/MS21/MS63)

FOR IN-VITRO DIAGNOSTIC USE

For Tube Testing

MEETS FDA POTENCY REQUIREMENTS

U.S. License Number: 1798

Package size

REF	802280100	VOL	5 mL	Seraclone® Anti-C (RH2)
REF	802346100	VOL	5 mL	Seraclone® Anti- \bar{c} (RH4)
REF	802336100	VOL	5 mL	Seraclone® Anti-E (RH3)
REF	802370100	VOL	5 mL	Seraclone® Anti-e (RH5)

Intended Use

For the determination of the Rhesus antigens C (RH2), E (RH3), \bar{c} (RH4), e (RH5) of red blood cells using the tube test.

Summary

More than 50 antigens belong to the Rhesus blood group system. The antigens C, \bar{c} , E and e, along with D, are the principle antigens of the Rh system. Although many other antigens have been identified, the antibodies associated with these 5 antigens are responsible for the majority of hemolytic transfusion reactions and cases of hemolytic disease of the fetus and newborn associated with the Rh system. For the determination of Rh phenotypes the C, c, E and e antigens on the red blood cells are tested with Anti-C (RH2), Anti- \bar{c} (RH4), Anti-E (RH3) and Anti-e (RH5). If the red blood cells carry only big C or little c (or E or e) the individual is treated as being homozygous for that particular antigen (allele). The most probable genotype can be presumed by determining the phenotype. The ethnic origin influences the genotype, which can be seen in the table.

Incidence of the More Common Genotypes in D+ Persons¹

Antigens Present	Genotype		Incidence (%)	
	DCE	Mod. Rh-hr	Whites	Blacks
D,C,c,e	DCE/ce	R ₁ r	31.1	8.8
	DCE/Dce	R ₁ R ₀	3.4	15.0
	Dce/Ce	R ₀ r'	0.2	1.8
D,C,e	DCE/DCE	R ₁ R ₁	17.6	2.9
	DCE/Ce	R ₁ r'	1.7	0.7
D,c,E,e	DcE/ce	R ₂ r	10.4	5.7
	DcE/Dce	R ₂ R ₀	1.1	9.7
D,c,E	DcE/DcE	R ₂ R ₂	2.0	1.3
	DcE/cE	R ₂ r''	0.3	<0.1
D,C,c,E,e	DCE/DcE	R ₁ R ₂	11.8	3.7
	DCE/cE	R ₁ r''	0.8	<0.1
	DcE/Ce	R ₂ r'	0.6	0.4
D,c,e	Dce/ce	R ₀ r	3.0	22.9
	Dce/Dce	R ₀ R ₀	0.2	19.4

Biotest Seraclone® Rhesus Blood Group Reagents are used to test for the presence or absence of the Rhesus antigens C, \bar{c} , D, E, e. Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Biotest Seraclone® Anti-C (RH2), Seraclone® Anti- \bar{c} (RH4), Seraclone® Anti-E (RH3) and Seraclone® Anti-e (RH5)

are used principally in the resolution of antibody problems or in family studies.

Principle of the Test

The test principle is hemagglutination. The antibodies in Seraclone® Anti-C (RH2), Seraclone® Anti- \bar{c} (RH4), Seraclone® Anti-E (RH3) and Seraclone® Anti-e (RH5) bind to the corresponding antigen on red blood cells and cause an antigen-antibody reaction visible as red blood cell agglutination.

Reagent

As the reactive components Seraclone® Anti-C (RH2), Seraclone® Anti- \bar{c} (RH4), Seraclone® Anti-E (RH3) and Seraclone® Anti-e (RH5) contain human monoclonal antibodies of the immunoglobulin class IgM. They are derived from cell culture supernatant and demonstrate the consistent specificity and reproducibility characteristic for monoclonal antibodies. Seraclone® Anti-E (RH3) does react with E^w.

Anti-C.

The antibodies are diluted in an isotonic saline solution containing bovine albumine

Anti- \bar{c}

The antibodies are diluted in a buffered saline solution containing macromolecular potentiator

Anti-E and Anti-e

Antibodies are diluted in a buffered protein solution containing macromolecular potentiators.

The following antibodies are produced using intermediate products produced for Biotest Medical Diagnostics GmbH in a shared manufacturing agreement with Millipore (UK) Ltd., 9 Fleming Road, Kirkton Campus, EH547BN, Livingston, UK; License Number 1721.

Seraclone® Anti-C (RH2) clone MS24 (IgM)

Seraclone® Anti- \bar{c} (RH4) clone MS33 (IgM)

Seraclone® Anti-E (RH3) clone MS260/MS12 (IgM/IgM)

Seraclone® Anti-e (RH5) clone MS16/MS21/MS63 (IgM/IgM/IgM)

Preservative: 0.1% sodium azide.

Precautions

- For In-vitro diagnostic use.
- Store at 2 to 8°C.
- Do not use beyond the expiration date.
- Do not use if turbid.
- Handle and dispose of reagents as potentially infectious
- Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
- Caution: This product contains Natural Rubber Latex Which May Cause Allergic Reactions.
- Warning: Contains sodium azide (NaN₃), which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.
- The bovine albumin used for the production of this reagent is purchased from BSE-free US sources, Boval Company L.P. in Cleburne, Tx, USA and Millipore in Kankakee, IL, USA.

Specimen Collection

Fresh samples of clotted, EDTA or citrate anticoagulated whole blood collected following general blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C.

Blood specimens exhibiting gross hemolysis or contamination should not be used.

Clotted samples or those collected in EDTA may be tested within ten days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiration date of the donor unit.

Materials

Materials provided

- Seraclone® Anti-C (RH2), Seraclone® Anti- \bar{c} (RH4), Seraclone® Anti-E (RH3) and/or Seraclone® Anti-e (RH5)

FOR REFERENCE USE ONLY: DO NOT USE in place of package inserts provided with each product.

Materials required but not provided

- Pipettes (drop volume 40 to 50 µl)
- Isotonic saline solution
- Negative Control (e.g. Biotest Seraclone® Control ABO+Rh [REF] 805171100)
- Glass tubes 10 x 75mm or 12 x 75 mm
- Serological Centrifuge
- Interval Timer
- Markers
- Optical aid (optional). The use of an optical aid for agglutination reading must be validated by the user.

Test Procedure

Tube test

1. Prepare a 3 to 5% suspension of red blood cells to be tested in isotonic saline.
2. Place one drop reagent into an appropriately labeled tube.
3. Add one drop of red blood cell suspension into the tube and mix.
4. Incubate at room temperature (20 to 24°C) for 5 minutes.
5. Centrifuge for 20 seconds at 800 -1000 x g.
6. Gently dislodge red blood cell button and observe for agglutination.
7. Record results

Stability of the Reaction

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

Quality Control

The reactivity of all Blood Grouping Reagents should be confirmed by testing with known positive and negative red blood cells on each day of use.

To confirm the reactivity or specificity of Biotest Monoclonal Rh Blood Grouping Reagents (Anti-C, Anti-E, Anti-c, Anti-e), each should be tested with antigen-positive (preferably from heterozygous individuals) and antigen-negative red blood cells, respectively. Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells. A negative control should be performed on samples testing positive with Anti-A, Anti-B, and Anti-D. Seraclone® Control ABO + Rh may be used.

Interpretation of results

Agglutination of the red blood cells is a positive result and indicates the presence of the corresponding antigen. No agglutination is a negative result and indicates the absence of the corresponding antigen.

An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual, 15th edition). Frequencies in the population are listed in the "Summary" section.

Limitations

- Samples with a positive direct antiglobulin test, cold agglutinins, or rouleaux formation may show false positive results in testing with monoclonal antibodies. Results on these samples must be interpreted with caution. False positive results or reaction suspected to be due to cold agglutinins should be resolved according to in-house procedures. It is recommended that an appropriate control be tested in parallel.
- The Anti-C clone MS24 gives only a weak positive reaction if the C and E antigens are located on one chromosome.
- Some conditions that may cause false positive results are:
 - Contamination of sample or reagents
 - Autoantibodies
 - Improper storage or preparation of red blood cells
 - Antibodies to antibiotics or other reagents
 - Cold Antibodies

Specific Performance Characteristics

Testing is performed in accordance with FDA recommended methods. The final release testing is performed according to the product specific SOPs. Each lot of Biotest blood group reagent is tested in the Quality control by package insert method against a panel of antigen positive red blood cells (heterozygous antigen expression and if possible weakened antigen expression) to insure suitable reactivity. The products meet FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs. For the product performance it is necessary to adhere to the recommended method in the instructions for use.

The performance of the Biotest Anti-C, Anti- \bar{c} and Anti-E, Anti-e was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

For Technical Support or further product information, contact Biotest Diagnostics Corporation at 800-522-0090.

Note

Each facility should verify the optimum spin time for the specific centrifuge in use.

Manual techniques are to be performed according to the manufacturer's instructions. Each deviation from these instructions is the sole responsibility of the user.

Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

Glossary of Symbols

Symbol	Definition	Symbol	Definition
	Batch Code		In vitro diagnostic medical device
	Caution, consult accompanying documents		Consult instructions for use.
	Manufacturer		Use by YYYY-MM-DD
	Contains sufficient quantity for <n> tests.		Catalog number
	Temperature limitation		Volume

Bibliography

1. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005.
2. Marion E. Reid, Christine Lomas-Francis, The Blood Group Antigen FactsBook, New York, NY: Academic Press, 2004.