Blood Grouping Reagent

Anti-C (RH2)
Seraclone® Human Monoclonal
(MS24)

Anti-¢ (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: 1845
Rx only

PACKAGE SIZE

[REF] 802280100 [VOL] 5 mL Seraclone® Anti-C (RH2)
[REF] 802346100 [VOL] 5 mL Seraclone® Anti-¢ (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), ¢ (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, ¢, 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-¢ (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+ Persons[1]

	Genotype	Genotype	Incidence (%)	Incidence (%)
Antigens Present	**DCE**	**Mod. Rh-hr**	**Whites**	**Blacks**
D,C,c,e	**DCe/ce**	**R_1r**	**31.1**	**8.8**
D,C,c,e	**DCe/Dce**	**R_1R_0**	**3.4**	**15.0**
D,C,c,e	**Dce/Ce**	**R_0r'**	**0.2**	**1.8**
D,C,e	**DCe/DCe**	**R_1R_1**	**17.6**	**2.9**
D,C,e	**DCe/Ce**	**R_1r'**	**1.7**	**0.7**
D,c,E,e	**DcE/ce**	**R_2r**	**10.4**	**5.7**
D,c,E,e	**DcE/Dce**	**R_2R_0**	**1.1**	**9.7**
D,c,E	**DcE/DcE**	**R_2R_2**	**2.0**	**1.3**
D,c,E	**DcE/cE**	**R_2r''**	**0.3**	**<0.1**
D,C,c,E,e	**DCe/DcE**	**R_1R_2**	**11.8**	**3.7**
D,C,c,E,e	**DCe/cE**	**R_1r''**	**0.8**	**<0.1**
D,C,c,E,e	**DcE/Ce**	**R_2r'**	**0.6**	**0.4**
D,c,e	**Dce/ce**	**R_0r**	**3.0**	**22.9**
D,c,e	**Dce/Dce**	**R_0R_0**	**0.2**	**19.4**

Bio-Rad Seraclone® Rhesus Blood Group Reagents are used to test for the presence or absence of the Rhesus antigens C, ¢, D, E, e. Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Bio-Rad Seraclone® Anti-C (RH2), Seraclone® Anti-¢ (RH4), Seraclone® Anti-E (RH3) and Seraclone® Anti-e (RH5) are used principally in the resolution of antibody problems or in family studies.

PRINCIPLES OF THE TEST
The test principle is hemagglutination. The antibodies in Seraclone® Anti-C (RH2), Seraclone® Anti-¢ (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
[IVD]
OBSERVABLE INDICATIONS
Do not use if markedly turbid.
Do not use damaged vials.

As the reactive components Seraclone® Anti-C (RH2), Seraclone® Anti-¢ (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 albumin.

Anti-¢
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 Bio-Rad 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-¢ (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 (NaN_3), 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 sourced from donor animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service inspectors to be disease free.
- Consult **downloads.bio-rad.com** to download the valid version of this instruction for use.

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, specimens should be stored at 2 to 8°C, citrated specimens (donor segments) may be stored at temperatures as low as 1°C.
Note: 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-¢ (RH4), Seraclone® Anti-E (RH3) and/or Seraclone® Anti-e (RH5)

Materials required but not provided
- Pipettes
- Isotonic saline or Phosphate Buffered Saline (PBS; pH of 7.2 +/-0.1)
- Negative control (e.g. Bio-Rad Seraclone® Control ABO+Rh [REF] 805171100)
- Glass tubes 10 x 75mm or 12 x 75 mm
- Serological centrifuge
- Interval timer
- Markers
- Agglutination viewer (optional).

TEST PROCEDURE
Tube test
1. Prepare a 3 to 5% suspension of red blood cells to be tested in saline.
2. Place one drop reagent into an appropriately labelled tube.
3. Add one drop (approx. 40 to 50µL) of red blood cell suspension into the tube and mix.

4. Incubate at room temperature (15 to 30°C) for 5 to 10 minutes.
5. Centrifuge for:
   a. 20 seconds at 800 to 1000 x g, or
   b. at a time and speed appropriate for the centrifuge calibration.
6. Gently dislodge red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however, microscopic examination is not recommended.
7. Record results.

STABILITY OF 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 Bio-Rad 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)[1].
Frequencies in the population are listed in the "Summary" section.

LIMITATIONS
- Samples with 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.
- The Anti-C clone MS24 gives only a weak positive reaction if the C and E antigens are located on one chromosome.
- Due to variants of the c antigen results could be false positive with the monoclonal Anti-C reagents.
- Rh32 positive red cells (C)D(e) and Cx red cells (C)CxD(e) show a weak C- and e-antigen expression and react weak positive or negative with the reagents anti-C and anti-e.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- In case of ambiguous results it is recommended to wash red blood cells at least 2 times.
- Very weak antigen expression may not be detected.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Mixed field reaction due to patients pre-transfusion history
  - Cross reactions with patient's medication (e.g. antibiotics)

SPECIFIC PERFORMANCE CHARACTERISTICS
Testing is performed in accordance with FDA recommended methods.
The final release testing is performed according to the product specific SOPs. As part of a release process each lot of Bio-Rad blood group reagent is tested according to the 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 Bio-Rad Anti-C, Anti-¢ 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 Bio-Rad Laboratories, Inc. at 800-224-6723.

NOTE
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
[LOT]	Batch Code	[IVD]	*In vitro* diagnostic medical device
!	Consult the instructions for use for important cautionary information such as warnings and precautions	I	Consult instructions for use
M	Manufacturer	e	Use by YYYY-MM-DD
s	Contains sufficient quantity for <n> tests	[REF]	Catalog number
t	Temperature limitation	[VOL]	Volume

Bibliography

1. John D. Roback, MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.
2. Marion E. Reid, Christine Lomas-Francis, The Blood Group Antigen FactsBook, New York, NY: Academic Press, 2004.

Key: Underline = Addition of changes ◄ = Deletion of text