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CE

Information for use

Rev. 001/05-2021

Description	REF
Anti-S Klon: MS-94 2 ml	24102
Anti-S Klon: MS-94 5 ml	24105

IN VITRO DIAGNOSTICUM

SUMMARY

In 1947 Walsh and Montgomery found Anti-S and named the associated antigen S. In the following, Race and Sanger described the inheritance according to Mendel's laws and a strong coupling to the neighboring genus MN.
Antibodies against the S blood group characteristic can lead to haemolyticus neonatorum disease (MHN) or haemolytic transfusion reactions. The dose effect is typical of the antigen.

INTENDED USE

Anti-S monoclonal (human IgM, clone MS-94) is used for the specific, qualitative detection of the corresponding antigen on erythrocytes and is suitable for slide, spot plate, microtiter plate and tube tests.
The test methods given are based on the principle of hemagglutination. After adding erythrocytes to the test reagents, a specific antigen-antibody reaction takes place if the corresponding antigen is present on the erythrocytes. This reaction can be visually recognized by the agglutination of the erythrocytes. If no agglutination occurs, this indicates a negative result and, taking into account the limitations of the test methods, indicates the absence of the corresponding antigen.

PRODUCT INFORMATION

The monoclonal anti-S test reagent is obtained from human hybridoma cell lines. The antibodies are suspended in a buffered 0.9% NaCl solution that contains bovine albumin (without stabilizer), EDTA and reagents that enable the cell button to be resuspended more easily after centrifugation. Preservative: Na azide (<0.1%).
All test reagents are used without further dilution / additives.
LOT and expiration date are on the vial label.

STORAGE

The test reagents can be used up to the expiry date stated on the label when stored at 2 ° C-8 ° C. After opening the test reagents for the first time, store them tightly closed at 2 ° C-8 ° C.

SPECIMEN COLLECTION AND PREPARATION

Blood samples should be collected in EDTA or citrate tubes according to standard medical procedures. The evaluation should be carried out as soon as possible after the blood collection. If the blood is not to be used immediately, the tubes should be stored at 2 ° C - 8 ° C. Blood samples showing hemolysis or microbial contamination should not be used for the test. Such blood tests can give incorrect results.
All blood samples are washed twice in 0.9% NaCl solution for the tube and microtiter plate test before use. Whole blood (35-45% erythrocyte suspension) is used for the slide test, whole blood or a 10% erythrocyte suspension in 0.9% NaCl solution for the spot plate test.

WARNINGS AND PRECAUTIONS

1. The reagents are intended for in vitro diagnostic laboratory use only
2. These reagents are designed for use by authorized operators trained in serological techniques.
3. These reagents are not intended for self-application.
4. Do not use these reagents past the expiration date.
5. Discard the content of damage vials.
6. Slight cloudiness does not affect the reactivity of the product.
7. The test sera contain <0.1% sodium azide as a preservative.
8. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
9. The test sera have been filtered through a 0.2µm capsule to reduce the bio-burden.
10. Once opened, the contents should be used up to the expiration date. Should it become cloudy or contaminated after opening, the contents should be discarded.
11. CE-Immundiagnostika GmbH cannot guarantee that human and animal raw materials are free from infectious agents, so the products should be used with caution.

DISPOSAL AND DECONTAMINIATION

For information on disposal of the reagent and decontamination of a spillage site, see the Material Safety Data Sheet, available on request from CE-Immundiagnostika GmbH.

CONTROLS AND ADVICE

1. Positive and negative control erythrocytes shall be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected reactions.
2. Since these reagents do not contain macromolecular potentiators, it is very unlikely that false positive or false negative reactions are caused with IgG coated cells.
3. 1 volume is approximately 35-45µl when using the vial dropper provided.
4. The reading and 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.
5. These reagents should be used only according to these instructions for use.

MATERIALS AND REAGENTS REQUIRED

- 0,9% NaCl-Lösung
- Glass test tubes
- Test Tube holder
- Test tube centrifuge
- Micro plate, plate shaker
- Spot plate
- Glass microscope slides
- Applicator stick
- Positive and negative control erythrocytes
- Chronometer

RECOMMENDED TECHNIQUES

A. METHOD: TUBE TEST

1. 2-4% erythrocyte suspension is prepared in 0.9% NaCl solution
2. 1 drop of antiserum and 1-2 drop/s of erythrocyte suspension are placed in a labelled test tube.
3. Mix well and centrifuge immediately for 1 min. at 400g (or at an alternative speed with an appropriate time).
4. Read the result immediately: loosen the erythrocyte pellet from the bottom of the tube by gently shaking it and read and record the agglutination strength macroscopically.

B. METHOD: MICRO PLATE TECHNIQUES

Pre-treatment of the microtiter plates (MTP):

MTP from different manufacturers / suppliers show different static properties, which can result in non-specific reactions of red blood cells and proteins. It is recommended that unused MTP be pre-treated prior to use to minimize red blood cell adhesion. MTP with a U-profile made of plastic are recommended.

1. Add 1 drop of 22% beef albumin (BSA) to each MTP well.

2. Mix by gentle agitation or on an MTP shaker so that the wells are evenly coated.
3. Let the MTP stand for at least 10 to 15 minutes at room temperature (18-25 ° C).
4. Drain the BSA and place the contents of the MTP wells in an appropriate waste container.
5. Rinse the MTP at least 10 times with tap water.
6. Then rinse the MTP twice with distilled or deionized water.
7. Tilt and blot the MTP to remove excess water.
8. Allow the MTP to dry before use.

Alternative methods are possible, provided they have been appropriately validated by the user.

PROCEDURE:

1. Prepare a 2 - 4% erythrocyte suspension in 0.9% NaCl solution. (Recommendation 2% suspension)
2. Pipette 30 µl of the corresponding test reagent into the marked MTP wells.
3. Add 30 µl of the prepared erythrocyte suspension to the MTP.
4. Mix MTP manually or on a shaker for 30 seconds.
5. Centrifuge the MTP for 1 min. At 400g (1500 rpm, or at an alternative speed with an adapted time). Shake the MTP briefly, if necessary with a shaker.

Record the result and reaction strength, positive and negative controls must be included. When using readers, these must be validated. The use of additional visual aids such as test reading mirrors or magnifying glasses can make reading easier.

C. METHOD: SLIDE TECHNIQUES

1. Whole blood is used for the slide test (35-45% erythrocyte suspension)
2. Put 1 drop of test reagent and one drop of red cell suspension on the slide.
3. Mix both drops thoroughly with a clean glass rod over an area of about 20x40mm.
4. Slowly move the slide back and forth
5. Read the result macroscopically after a maximum of 2 minutes and record it.
6. In the event of improper handling or an incubation time that is too long, drying artifacts may occur; the test must be discarded.

D. METHOD: SPOT PLATE TECHNIQUES

1. Whole blood (35-45% erythrocyte suspension) or a 10% erythrocyte suspension in 0.9% NaCl solution is used for the spot plate test.
2. Put 1 drop of test reagent + 1 drop of erythrocyte suspension on the spot plate.
3. Mix both drops thoroughly with a clean stirring rod.
4. Incubate for 5-10 min. At room temperature.
5. Read the result macroscopically and record it.

Improper handling or an incubation time that is too long can lead to drying artifacts.

INTERPRETATION OF THE RESULTS

1. **Positive:** The agglutination of the erythrocytes indicates the presence of the antigen to be determined on the erythrocytes. Please note the limits of the test method (see below).
2. **Negative:** no agglutination indicates the absence of the antigen to be determined on the erythrocytes. Please note the limits of the test method (see below).

Discrepancies: If the results of the antigen determination on the erythrocytes do not agree with the determination of the alloantibodies of a blood group, further tests must be carried out.

LIMITATIONS

1. Blood that is not freshly drawn can lead to poorer results. Blood that is no more than two days old is recommended.
2. The reaction strength should be 2+ to 4+, weaker reactions should be checked again with a longer incubation time.
3. False positive or false negative results can be caused by:
   - Contamination of the material to be tested
   - Incorrect storage, incorrect erythrocyte concentration, incorrect incubation time, incorrect temperature
   - Incorrect centrifugation
   - Deviations from the recommended methods
4. Patients with certain diseases can show false positive / negative reactions. Umbilical cord blood with Wharton's jelly can react with false positive results.
5. The enzyme treatment of erythrocytes can lead to the destruction of the S-antigen.
6. At room temperature> +20 ° C, it is recommended to cool the reagent to + 2 ° C to + 8 ° C beforehand.
7. **According to the Hemotherapy Guideline, Chapter 4.4.8, 2017, two different test reagents must always be used to determine the S-antigen.**

STABILITY OF THE REACTIONS

1. Read all tube and microplate tests straight after centrifugation.
2. Slide tests should be interpreted within 2 minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
3. Tests must be considered invalid if they have been performed at temperatures other than those recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The antisera were tested with the method before the release, which is based on the GTS guideline, since no specifications are required.
2. Each LOT of monoclonal antisera is tested against a panel of antigen-positive erythrocytes prior to release to ensure good reactivity.
3. The specificity of monoclonal antibodies is demonstrated using panels with antigen-negative erythrocytes.
4. Erythrocytes or whole blood washed twice in 0.9% saline solution are used in quality control.
5. Tested on over 500 samples with sensitivity and specificity of 100%.

DISCLAIMER

1. The user is responsible for the performance of the reagents by any method other than those recommended.
2. Any deviations from the Recommended Techniques should be validated prior to use.

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3. Race RR, Sanger R. Blood groups in man. 6th ed. Oxford, Blackwell Scientific Publications; 1975:336.
4. Daniels G. Human Blood Groups, 2002, 2nd Edition, Blackwell Science Publications

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24102 Anti-S 2 ml
24105 Anti-S 5 ml

EXPLANATION OF THE SYMBOLS

LOT	Batch No.	IVD	In-vitro Diagnosticum
REF	Product-Code	+2°C +8°C	Store at + 2 °C bis + 8 °C
	Expiry date		Manufacturer
	Information for use		

ARTICLE NUMBERS

REF Product Clone	Possible shipping quantity
24102 Anti-S Clone: MS-94	1 x 1 x 2 ml 5 x 1 x 2 ml 10 x 1 x 2 ml 50 x 1 x 2 ml
24105 Anti-S Clone: MS-94	1 x 1 x 5 ml 5 x 1 x 5 ml 10 x 1 x 5 ml 50 x 1 x 5 ml