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CE

## Information for use

**Rev. 002/06-2021**

| Description | REF |
|---|---|
| **Anti-N** Klon: 1422C7 2 ml | 23102 |
| **Anti-N** Klon: 1422C7 5 ml | 23105 |

*IN VITRO* DIAGNOSTIC MEDICAL DEVICE

### SUMMARY

Landsteiner and Levine discovered the MN system in 1927 and produced anti-M and anti-N serums that detected the traits M and N by immunizing rabbits. In the Caucasian population, M is 78.7% and N is 70.2%.
Antibodies against the N blood group characteristic are predominantly cold reactive antibodies.. The dose effect is typical of the antigen.

### INTENDED USE

Anti-N monoclonal (murine IgG, clone 1422C7 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 agglutination does not occur, 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-N test reagent (IgG, 1422C7) is obtained from murine 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.
The 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 should be drawn aseptically in EDTA or citrate tubes. The evaluation should be carried out as soon as possible after the blood sample has been drawn. 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. The reagents may only be used by authorized and trained specialist personnel.
3. The test reagents are not intended for personal use.
4. After the expiry date, the test sera may no longer be used
5. Damaged vials must not be used
6. Slight cloudiness does not affect the reactivity of the product.
7. The test reagents contain <0.1% sodium azide as a preservative.
8. Wear protective clothing such as a gown and disposable gloves when using the products
9. The test sera were filtered through a 0.2 µm membrane in order to reduce the bacterial load.
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 OF REAGENT AND DEALING WITH SPILLAGES

For disposal of the test sera or decontamination in the event of spillage, please request the safety data sheet from CE-Immundiagnostika GmbH.

### CONTROLS AND ADVICE

1. Positive and negative control erythrocytes must be included with each experiment. If the controls do not show the expected results, the test batch should be discarded.
2. 1 drop from the pipette vial corresponds to 35-45µl.
3. Only authorized specialists are allowed to read and evaluate the results.
4. The test reagents may only be used as described here.

### REQUIRES MATERIAL AND REAGENTS

- 0.9% NaCl solution
- Glass tube
- Tube holder
- centrifuge
- microplate, shaker
- spot plate
- Glass microscope slide
- Applicator stick
- Positive and negative control erythrocytes
- chronometer

### RECOMMENDED TECHNIQUES

#### A. METHODE: TUBE TECHNIQUE

1. Prepare a 2-4% suspension of erythrocytes in a 0.9% NaCl solution.
2. Place 1 volume of test reagent and 1 volume of test suspension of test erythrocytes in a labelled test tube.
3. Mix thoroughly, incubate 10 – 15 minutes at room temperature and centrifuge at 400g for 1 minute (at 1,500 rcf or for a suitable alternative time and force).
4. Read the result immediately: Gently agitate the tube to dislodge the erythrocyte pellet from the bottom of the test tube and read macroscopically for agglutination; record result.

#### B. METHODE: MICROPLATE TECHNIQUE

**Preparation of the microplates:**
Microplates made by different manufacturers / suppliers have different static properties that may give rise to non-specific red blood cell and protein reactions. It is advisable to pre-treat unused microplates prior to use to reduce the build-up of red blood cells to a minimum. We recommend using “U” wells made from plastic material.

1. Place 1 volume of 22% bovine albumin (BSA) in the appropriate wells.
2. Mix thoroughly, gently agitating or using a microplate shaker, to ensure that wells are covered evenly.
3. Incubate at room temperature (18-25°C) for no less than 10 and no more than 15 minutes.
4. Drain the BSA and discard the content of the wells in a suitable waste disposal container.
5. Flush the microplate at least 10 times with tap water.
6. Then rinse the microplate twice with distilled or deionized water.
7. Tilt and dab the microplate to remove excess water.
8. Allow the microplate to dry prior to use.

Alternative techniques may be used provided that they have been validated by the user.

**Procedure:**

1. Prepare a 2 - 4% suspension of test erythrocytes in a 0.9% NaCl solution. (Recommendation: 2% suspension)
2. Using the vial dropper, place 30µl of the appropriate test reagent into the marked wells of the microplate.
3. Add 30µl of the previously prepared suspension of test erythrocytes to the microplate.
4. Mix for 30 seconds, either manually or using a shaker.
5. Centrifuge the microplate for 1 minute at 400g (at 1,500 rcf or for a suitable alternative time and force). Briefly agitate the microplate, using a shaker, if necessary.

Record the result and the reaction intensity, testing positive and negative control erythrocytes in parallel. Reading devices, if used, must have been validated. Using additional visual expedients such as, for instance, mirrors or magnifying glasses may facilitate reading the results.

**C. METHODE: SLIDE TECHNIQUE**

1. Using whole blood, prepare a 35-45% suspension of test erythrocytes.
2. Place 1 volume of test reagent and 1 volume of whole blood on a slide.
3. Using a clean applicator stick, thoroughly mix both volumes over an area of about 20x40mm.
4. Slowly move the slide back and forth.
5. Read macroscopically after no more than 2 minutes and record.
6. Incorrect handling or excessive incubation time may lead to drying-induced artefacts, and the test must be considered invalid.

**D. METHODE: SPOT PLATE TECHNIQUE**

1. Using whole blood, prepare a 35-45% suspension of test erythrocytes or a 10% suspension of test erythrocytes in a 0.9% NaCl solution.
2. Place 1 volume of test reagent + 1 volume of suspension of test erythrocytes on a spot plate.
3. Using a clean applicator stick, mix both volumes thoroughly.
4. Incubate at room temperature for 5-10 minutes.
5. Read macroscopically and record.

Incorrect handling or excessive incubation time may lead to drying-induced artefacts.

**INTERPRETATION OF TEST RESULTS**

1. **Positive:** Agglutination of the test erythrocytes indicates, within accepted limitations of test procedure (see below), the presence of the appropriate antigen on the test erythrocytes.
2. **Negative:** No agglutination of the test erythrocytes indicates, within accepted limitations of test procedure (see below), the absence of the appropriate antigen on the test erythrocytes.

**LIMITATIONS**

1. Stored blood may give weaker reactions than fresh blood.
2. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper storage, erythrocyte concentration, incubation time or temperature
   - Improper or excessive centrifugation
   - Deviation from the recommended techniques
3. Specimens displaying haemolysis or microbial contamination must not be tested.
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 N-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 N-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 liable if any other than the recommended one is used.
2. Any deviations from the recommended test method must be validated prior to use.

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4. Daniels G. Human Blood Groups, 2002, 2nd Edition, Blackwell Science Publications

**INDEX of SYMBOLS**

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**Rev. 002/06-2021**

Anti-N 23102
Anti-N 23105

| | | | |
|---|---|---|---|
| LOT | lot | IVD | In-vitro Diagnosticum |
| REF | Product code |  | Storage between + 2 °C bis + 8 °C |
|  | Expiry date |  | manufacturer |
|  | Consult instruction for use (insert) | | |

**ARTICLE NUMBER**

| REF Product Clone | Quantity |
|---|---|
| 23102<br>Anti-N<br>Klon: 1422C7 | 1 x 1 x 5 ml<br>5 x 1 x 5 ml<br>10 x 1 x 5 ml<br>50 x 1 x 5 ml |
| 23105<br>Anti-N<br>Klon: 1422C7 | 1 x 1 x 5 ml<br>5 x 1 x 5 ml<br>10 x 1 x 5 ml<br>50 x 1 x 5 ml |