EN INSTRUCTIONS FOR USE

Anti-Jk^a (Kidd^a), -Jk^b (Kidd^b) **monoclonal (IgM)** CE 0123

Anti-N, -S, -P_1 **monoclonal (IgM)** CE

Anti-M **monoclonal (IgG)** CE

Electronic Instructions for use see www.bag-diagnostics.com

Product		Clone	REF
Anti-Jk^a monoclonal (IgM)	2 ml	MS15	6770
Anti-Jk^b monoclonal (IgM)	2 ml	MS8	6772
Anti-M monoclonal (IgM)	5 ml	M-11H2	67622
Anti-N monoclonal (IgM)	5 ml	1422C7	67611
Anti-S monoclonal (IgM)	2 ml	MS94	6849
Anti-P_1 monoclonal (IgM)	5 ml	650	6734

FOR IN VITRO DIAGNOSTIC USE

1. Description of products

Anti-Jk^a, Anti-Jk^b, Anti-S are made from monoclonal human IgM antibodies and Anti-N, Anti-P_1 are made from monoclonal mouse IgM antibodies. Anti-M is made from monoclonal mouse IgG antibodies. The clone numbers are given on the labels of the test reagents.
The test reagents aid in the detection of the corresponding antigens on red blood cells and are suitable for the test tube procedure.
NaN_3 (< 0.1%) is added to the test reagents as a preservative. The monoclonal antibodies are suspended in a buffered isotonic solution.
Anti-Jk^a and Anti-Jk^b contain Bovine albumin (BSA) as a reaction enhancer.

2. Principle of the test

The testing method indicated is based on the principle of hemagglutination. A specific antigen-antibody reaction takes place once red blood cells are added to the monoclonal test reagents if the corresponding antigen is present on the red blood cells. This reaction is visibly recognizable by the agglutination of the red blood cells. If no agglutination takes place, this indicates a negative result and, allowing for the limitations of the testing method, the absence of the corresponding antigen.

3. Storage and stability

Store the test reagents at 2...8°C. Do not freeze! Allow the reagents to come to room temperature (< 20°C) before use and store again at 2…8°C after use.

Once they have been opened the first time, the test reagents may be used up to the expiration date indicated on the label if the specified storage conditions are observed. Do not use the reagents past the expiration date indicated on the label. Do not use obvious turbid test reagents.

4. Preparation of samples

The blood samples should be collected according to the customary medical procedure. Blood samples with and without anti-coagulants (EDTA, citrate) are suitable for testing. If possible, use fresh samples and not hemolytic and/or contaminated samples! Testing should take place without delay. If this is not possible, store the sample at 2…8°C.
The strength of positive reactions depends on the age of the used blood. If red blood cells are stored for too long before testing, the red blood cell antigens may change, which can lead to weakened reactions. Enzyme treatments of red blood cells may lead to destruction of S-antigens and are to be excluded. To avoid unspecific reactions with Anti-N the test cells (e.g. Panocell) are to be suspended in isotonic NaCl solution before use, as recommended by the manufacturer (see 9. Important Notes/Limitations of the Method).

5. Additional materials required

Isotonic NaCl solution (isotonic saline)

Test tubes (75 x 12 mm)

Test tube rack

Single-use Pasteur pipettes

Centrifuge

Red blood cells of known phenotype

6. Test procedure

Tube test

1. Wash the red blood cells (patients / test red blood cells) to be examined at least once and then make a suspension of 2 - 3% in isotonic NaCl solution.
2. Mix 1 drop of monoclonal test reagent and 1 drop of the red blood cell suspension (Anti-M: 1-2 drops of red blood cell suspension) in a labeled test tube.

Anti-Jk^a, Anti-Jk^b:	Incubate for 5 - 15 minutes at room temperature, especially at 2…8°C
Anti-S, Anti-P_1	Incubate for 10 - 15 minutes at room temperature.
Anti-M:	Incubate 5 minutes at room temperature.
Anti-N:	No incubation, centrifuge immediately.

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3. Centrifuge Anti-S, Anti-P_1, Anti-Jk^a, Anti-Jk^b, Anti-N 1 minute at 400 x g (1500 rpm)

   and

   Anti-M 1 minute at 180-270 x g (approx.1000 rpm)

   or at an alternative rpm with an appropriate time adjustment.

4. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.

Comments: Do not examine the test microscopically.

Red blood cells that are positive with regard to the respective antigen (preferably heterozygote cells), and red blood cells that are negative with regard to the respective antigen, as well as a negative control for monoclonal test reagents and an auto-control to test for autoagglutination must also be tested as controls.

The determination of the antigens should be carried out with at least 2 different test reagents. When using two monoclonal test reagents, two different clones should be used, if possible.

7. Interpretation of the results

An agglutination of the red blood cells with the test reagent indicates the presence of the corresponding antigen.

If there is no agglutination of the red blood cells with the test reagent, this indicates the absence of the corresponding antigen.

The test results cannot be evaluated if there is no agglutination with the known positive red blood cell suspension, or if agglutination occurs with the known negative red blood cell suspension or the negative control for monoclonal test reagents or the auto-control.

If discrepant results occur with two different test reagents when determining the antigen, the determination must be repeated with another test method and/or an additional test reagent.

The limitations of the method must be considered when interpreting the results (see 9. Important Notes/Limitations of the Method).

8. Stability of reactions

All test results must be interpreted immediately once the test is performed.

9. Important Notes/Limitations of the Method

1. The test reagents are suitable for in vitro diagnostic use only and may only be used by trained, qualified personnel.
2. In rare cases, spontaneous and non-specific agglutinations may occur with red blood cells loaded with immunoglobulins in vivo. In such instances similar phenomena would most likely occur in blood grouping tests of other blood group systems as well. Therefore, as a control, a negative control for monoclonal test reagents and an autologous patient serum should always be tested as well. If the control tests also show a positive reaction, the result of the blood type determination cannot be interpreted.

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3. Suspensions of unwashed red blood cells in plasma or serum promote false positive reactions such as those associated with rouleaux formation or autoantibodies. The use of well-washed red blood cells can reduce the occurrence of such false positive reactions.
4. In the serum/plasma of the patient, soluble antigens that may be present can be adsorbed on the red blood cell surface, or they can neutralize antibodies targeted against the corresponding antigen. The use of well-washed red blood cells can prevent the occurrence of such false positive reactions.
5. Insufficient cell concentration, reading the results of the test too late, agitating the red blood cell sediment too strongly, and other deviations from the indicated testing procedure can lead to weaker or false negative results.
6. The test reagents should not be used for the testing of enzyme-treated red blood cells. Particularly the S-antigen may be destroyed by enzyme treatment.
7. Hemolytic and/or contaminated samples should not be used.
8. The strength of positive reactions depends on the age of the used blood.
9. False negative results or unexpected weak reactions may be caused by storing the red blood cells for too long and/or under inappropriate conditions and/or by a cell concentration that is too low.
10. False negative or false positive results can result from inappropriate techniques, incorrect centrifugation or incubation, dirty tubes, incorrect pH of the isotonic NaCl solution and/or contaminated materials and samples.
11. A microbial contamination of the test reagents must be absolutely avoided because this shortens the shelf life of the products and can lead to false results.
12. In presence of rare variants of antigens unexpected results may occur. For example the rare determinant M^e is also detected by some monoclonal anti-M sera, which can contain beside the M- a Henshaw- component (present in Central Africans with a frequency of 2,3 %).
13. False weak reactions (+/- or 1+) can occur with Anti-N monoclonal, clone 1422C7, by MMS+ antigen constellation. In most cases the agglutination dissolves after a short time.
14. In the case of detecting the N-antigen, unspecific reactions can occur due to reaction enhancer. Therefore test cells (e.g. Panocell) should be washed with isotonic saline and suspended in isotonic saline as stated by the manufacturer.
15. Light cloudiness does not influence the reactivity of the product.
16. Use the test reagent without any supplements.
17. At a room temperature above 20°C the M-, N- and S-test reagents and test cells should be cooled at 2…8°C before use.
18. No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended.
19. Deviation from the recommended Instructions for Use may result in less than optimal product performance. User-defined deviations such as modifications of test procedures, serum dilution for use in automat or cards, freezing of serum on microtiter plates etc. may require validation by the user.
20. Whether transfusions or transplantation have taken place should always be taken into consideration when interpreting the results. Any history of transfusions and/or

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transplantation, as well as the patient's medication history, should be taken into consideration when interpreting results.

21. Do not use mouse monoclonal reagents in direct antiglobulin tests with anti-human-globulin reagents.

10. Warnings and instructions for disposal

Materials of human origin used in the manufacture of the test reagents were tested for HBsAg and antibodies to HIV and HCV. Only negative material was used for manufacture. Bovine albumin resp. corresponding raw material is sourced from supervised BSE-free cattle herds. In spite of this, all materials of biological origin used for the test should be regarded as potentially infectious since no testing method can detect all infectious pathogens. Therefore, appropriate safety precautions are recommended when handling biological materials (do not pipette using the mouth; wear protective gloves when performing the test; disinfect hands after testing).

Biological materials must be deactivated before disposal (e.g., by autoclaving). Single-use materials must be autoclaved or incinerated after use.
Spills of potentially infectious material should be removed without delay with an absorbent paper towel and the contaminated area disinfected with an appropriate disinfectant or 70% ethanol. Materials used for the removal of spills must be deactivated before disposal (e.g., by autoclaving).

The test reagents contain as preservative < 0.1% NaN_3. A concentration of < 0.1% NaN_3 is not considered to be a harmful concentration. Nevertheless avoid contact with the skin and mucous membranes. The copper and lead used in some pipe systems can form explosive salts with azide. The amounts of azide contained in the reagents are small; nevertheless, copious amounts of water should be used for rinsing afterwards when disposing of azide-containing materials.

Disposal of all samples, unused reagent and waste should be in accordance with country, federal, state and local regulations.

A Material Safety Data Sheet (MSDS) is available to download at www.bag-diagnostics.com .

11. References

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Explanation of symbols used on Labelling	
IVD	For in vitro diagnostic use
	Manufacturer
	Storage temperature / Temperature limitation
LOT	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
MONOCL | IGM	Monoclonal IgM
MONOCL | IGG	Monoclonal IgG
CLONE	Clone
ORIG | HUM	Origin: human
ORIG | MURIN	Origin: murine
CONT | NaN_3	Contains Natriumazide
TIT	Titer

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Instructions for use in other languages see:
http://www.bag-diagnostics.com
or phone +49 (0) 6404-925-125

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