EN

INSTRUCTIONS FOR USE

Anti-Le^a (Lewis^a)

Anti-Le^b (Lewis^b)

monoclonal (IgM)

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

Product		Clone	REF
Anti-Le^a monoclonal (IgM)	5 ml	LEA2	67311
Anti-Le^b monoclonal (IgM)	5 ml	LEB2	67321

FOR IN VITRO DIAGNOSTIC USE

1. Description of product

Anti-Le^a (clone **LEA2**) and Anti-Le^b (clone **LEB2**) are made from monoclonal mouse IgM antibodies. The clone numbers are given on the labels of the test reagents.
Anti-Le^a and Anti-Le^b monoclonal (IgM) aid for the detection of the corresponding antigens on human red blood cells and are suitable for the test tube procedure.
NaN_3 (< 0.1%) is added to the test reagents as a preservative.

2. Principle of the test

The testing method indicated is based on the principle of hemagglutination. Incubation of test red cells with the test reagent will result in a specific antigen-antibody reaction if the corresponding antigen is present on the test red cells. Visible detection of this reaction is demonstrated by agglutination of the cells. No agglutination indicates a negative test result, and within the accepted limitations of the test procedure, indicates 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 and no signs of contamination are observed. Do not use the reagent after the expiry date printed on the label. Do not use contaminated reagents!

BAG Diagnostics GmbH
Amtsgerichtsstr. 1-5
35423 Lich/Germany
Tel.: +49 (0) 6404 / 925 - 100
Fax: +49 (0) 6404 / 925 - 460
www.bag-diagnostics.com
info@bag-diagnostics.com

Ordering:
Tel.: +49 (0) 6404 / 925 - 450
Fax: +49 (0) 6404 / 925 - 460
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Customer Service:
Tel.: +49 (0) 6404 / 925 - 125
Fax: +49 (0) 6404 / 925 - 421
service@bag-diagnostics.com

4. Preparation of samples

The blood samples should be collected by approved medical procedure. Blood samples with and without anticoagulants (EDTA, citrate) are suitable for testing. Do not use hemolytic samples! Testing should take place without delay whenever possible. If this is not possible, store blood samples at 2...8°C.
If red cells are stored for too long before testing, the red blood cell antigens may change, which can lead to weakened reactions (see 9. Important Notes/Limitations of the Method).

5. Additional materials required

0.9% NaCl solution (isotonic saline)

PBS (Phosphate-buffered saline), pH 6.8 – 7.2, or LISS

Test tubes (75 x 12 mm), especially of glass

Tube rack

Single-use Pasteur pipettes

Centrifuge

Red blood cells of known phenotype

6. Test procedure (Tube test)

1. Wash the red cells to be examined at least once in cold isotonic saline and then prepare a 2 – 3% suspension of test red cells in isotonic saline or PBS, pH 6.8 – 7.2 (alternative: prepare a 1.5 – 2% suspension of test red cells in LISS).
2. Mix 1 drop of monoclonal test reagent and 1 drop of the test red cell suspension in a labeled test tube and incubate at room temperature (< 20°C) for 10 - 15 minutes.
3. Centrifuge 1 minute at 400 x g (1500 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 cells that are positive with regard to the respective antigen, and red 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 auto-agglutination 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

Agglutination of test red cells with the test reagent indicates the presence of the corresponding antigen (within the accepted limitations of the test procedure).
No agglutination of test red cells with the test reagent indicates the absence of the corresponding antigen (within the accepted limitations of the test procedure).

If no agglutination occurs with the test red cells known to be positive for the antigen or if agglutination occurs with the test red cells known to be negative for the antigen or with the

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negative control for monoclonal test reagents or with the auto-control the test results should not be interpreted. If different test results occur with two different test reagents, repeat the determination of the antigen with an other test method and/or an other test reagent.

Pay attention to the limitations of procedure and important directions (s. 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 designed for in vitro diagnostic use only and should be used only by properly trained, qualified personnel.
2. The test reagents should not be used for the testing of enzyme-treated red cells.
3. Hemolytic samples should not be used.
4. The strength of positive reactions depends on the age of the used blood. This concerns in particular EDTA and native blood. Better results are achieved with freshly drawn blood samples.
5. Clone LEB2 has anti-Leb H specificity and react stronger with cells of group O phenotype. The clone react with A_1 and A_1B red cells but in some cases may exhibit a weaker reaction.
6. Umbilical cord red cells do not express sufficient Lewis antigen to be agglutinated by the test reagents. Therefore these cells are typed as Le (a-b-).
7. Light cloudiness does not influence the reactivity of the product.
8. In rare cases, spontaneous and non-specific agglutinations may occur with red cells coated 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.
9. Suspensions of unwashed red cells in plasma or serum promote false positive reactions such as those associated with rouleaux formation or autoantibodies. The use of well washed red cells can reduce the occurrence of such false positive reactions.
10. Soluble antigens that may be present in the serum/plasma of the patient can be adsorbed on the red cell surface, or they can neutralize antibodies targeted against the corresponding antigen. The use of well washed red cells can prevent the occurrence of such false positive reactions.
11. At a room temperature above 20°C both test reagents and test red cells should be cooled at 2…8°C before use.
12. Reading the results of the test too late, agitating the red cell sediment too strongly, and other deviations from the indicated testing procedure can lead to weaker or false negative results.
13. False negative results or unexpected weak reactions may be caused by storing the red cells for too long and/or under inappropriate conditions and/or by a cell concentration that is too low.

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14. False negative or false positive results can result from inappropriate techniques, incorrect centrifugation or incubation, dirty tubes, incorrect pH of solutions and/or contaminated materials and samples.
15. Do not use mouse monoclonal reagents in direct antiglobulin tests with anti-human-globulin (AHG) reagents.
16. A microbial or chemical contamination of the test reagents must be absolutely avoided because this shortens the shelf life of the product and can lead to false results.
17. 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.
18. 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.
19. Whether transfusions or transplantations have taken place should always be taken into consideration when interpreting the results. Any history of transfusions and/or transplantations, as well as the patient's medication history, should be taken into consideration when interpreting results.

10. Warnings and instructions for disposal

All used biological material should be handled as potentially infectious, because no test method can guarantee that material derived from biological sources are free from infectious agents. When handling biological material appropriate safety precautions are recommended (Do not pipette by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

The test reagents contain NaN_3 as a preservative. The reagents contain < 0.1% NaN_3 which is not considered to be a harmful concentration. Nevertheless avoid contact with the skin and mucous membranes. The copper and lead used in some plumbing systems can react with azides to form explosive salts. The quantities of azide used in this reagent are small; nevertheless when disposing of azide-containing materials, they should be flushed away with a large volume of water.

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 .

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11. References

Issitt PD, Anstee DJ. Applied Blood Group Serology. 4th Edition. Montgomery Scientific. Durham SC. 1998

Technical manual of the American Association of Blood Banks, 18th ed., 2014

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
CLONE	Clone
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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