

**EN**      **INSTRUCTIONS FOR USE****Anti-H monoclonal (IgM)****Clone: 10934C11**Electronic instructions for use see [www.bag-diagnostics.com](http://www.bag-diagnostics.com)**REF**    **6815**      **1 x 5 ml****FOR IN VITRO DIAGNOSTIC USE****1. Product description**

Anti-H monoclonal is prepared from monoclonal mouse IgM antibodies (Clone: 10934C11). It is used for the detection of H substance on human red blood cells and is designed for use in tube test. Anti-H is used for the determination of A-subgroups, since it reacts with erythrocytes of blood group A<sub>2</sub> and weak A-variants and reacts only weakly or not with erythrocytes of blood group A<sub>1</sub>.

For stabilization the diluent used for this reagent contains bovine albumine. The test reagent contains < 0.1% NaN<sub>3</sub> as preservative.

**2. Biological principle of the test**

The test performed with this blood grouping reagent is based on the principle of hemagglutination. Incubation of test red cells with Anti-H monoclonal will result in a specific antigen-antibody reaction when the corresponding H substance is present in sufficient quantities on the test cells. Visible detection of this reaction is indicated by agglutination of the cells.

**3. Storage and Shelf Life**

Store Anti-H monoclonal at 2...8°C. Do not freeze! After opening the bottle the test reagent can be used until the expiry date printed on the label under appropriate storage conditions. Do not use the reagent after the expiry date indicated on the label.

**4. Specimen preparation**

Blood samples should be collected by approved medical procedure. Blood samples with or without anticoagulant (EDTA, citrate) are suitable for testing. Do not use haemolytic samples.

Testing should take place without delay to minimize the risk of false results due to potential contaminations or improper storage (see 9. Important Notes/Limitations of the Method). If this is not possible, store blood samples at 2...8°C.

## **5. Additional Materials Required**

Isotonic NaCl solution (isotonic saline)  
Test tubes (75 x 12 mm)  
Test tube rack  
Disposable Pasteur Pipettes  
Centrifuge  
Red blood cells of known phenotype

## **6. Test procedure**

### **Tube test**

1. Wash the red cells to be investigated at least once in isotonic saline and then prepare a 2 – 3% suspension of test red cells in isotonic saline.
2. Mix 1 drop of monoclonal test reagent and 1 drop of the test red cell suspension in a labeled test tube.
3. Incubate for 10 - 15 minutes at room temperature.
4. Centrifuge 1 minute at 400 x g (1500 rpm) or at an alternative rpm with an appropriate time adjustment.
5. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.

**Directions:** Red blood cell suspensions known to be A<sub>1</sub> positive and known to be A<sub>2</sub> positive and a patient control should always be included in the test.

Use two different Anti-H test reagents to determine the A subgroups and always secure the test result using an Anti-A<sub>1</sub> test reagent and a test reagent without antibodies (negative control for monoclonal test reagents). Applying two monoclonal test reagents two different clones should be used.

## **7. Interpretation of test results**

A strong agglutination of red cells of blood group A with Anti-H monoclonal and no agglutination with the Anti-A<sub>1</sub> test reagent refer to the phenotype A<sub>2</sub> or a weak A variant. No agglutination or a weak reaction of red cells of blood group A with Anti-H monoclonal and a strong reaction with the Anti-A<sub>1</sub> test reagent refer to the phenotype A<sub>1</sub>.

If no strong agglutination occurs with the test red cells known to be A<sub>2</sub> positive or if a strong agglutination occurs with the test red cells known to be A<sub>1</sub> positive or the patient control and/or the test reagent without antibodies shows a positive reaction the test results should not be interpreted.

If discrepant test results occur with different test reagents, repeat the determination of the A subgroups with another test method and/or another test reagent (e.g. BAGene ABO-TYPE or BAGene ABO-TYPE variant).

When testing with Anti-H test reagents it has to be considered that red blood cells of blood group 0 carry the most H substance and show the strongest reaction. In descend sequence follow weak A variants >A<sub>2</sub>>A<sub>2</sub>B>B>A<sub>1</sub>>A<sub>1</sub>B. For this reason red blood cells of blood groups B, A<sub>1</sub> and A<sub>1</sub>B can also react with Anti-H. In case of A<sub>2</sub>B, A<sub>2</sub> is weakened in presence of B. Thus cause decreased reactions with Anti-H test reagents. The very rare Bombay phenotype carries no H substance and does not react with Anti-H.

Pay attention to the limitations of procedure and important directions (s. 9. Important Directions/Limitation of the method).

### **8. Stability Of The Reaction**

All test results should be interpreted immediately upon completion of the test.

### **9. Important directions / Limitations of the method**

1. The test reagent is designed for in vitro diagnostic use only and should be used only by well trained, qualified personnel.
2. The determination of A subtypes with this test procedure is not possible in neonates since these antigens are not yet expressed completely.
3. The strength of positive reactions depends on the age of the used blood. Prolonged and/or inappropriate storage conditions of the red cells can cause unexpectedly weak or false negative results.
4. Delays in reading tests, overvigorous resuspension of red cell buttons, insufficient cell concentration, unclean test tubes, incorrect saline pH, contaminated materials and samples or other deviations from the specified test performance may result in false negative or false positive test results.
5. A microbial or chemical contamination of the test reagent must be prevented because this shortens the shelf life of the product and cause erroneous results.
6. Light cloudiness does not influence the reactivity of the product.
7. No general 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.
8. Previous transfusions or transplantations should always be considered 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.
9. 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.
10. Do not use mouse monoclonal reagents in direct antiglobulin tests with anti-human-globulin reagents.

### **10. Warnings and Precautions**

All used biological material like the test reagent should be handled as potentially infectious. 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 reagent contains  $\text{NaN}_3$  as a preservative. The reagent contains  $< 0.1\%$   $\text{NaN}_3$  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](http://www.bag-diagnostics.com).

## 11. References

Applied Blood Group Serology, PD Issitt and DJ Anstee, 4<sup>th</sup> Edition, Montgomery Scientific, Durham SC, 1998

Technical manual of the American Association of Blood Banks, 18<sup>th</sup> ed., 2014

Explanation of symbols used on Labelling	
	For in vitro diagnostic use
	Manufacturer
	Storage temperature / Temperature limitation
	Batch code
	Use by
	Catalogue number
	<i>Consult instructions for use</i>
	<i>Monoclonal IgM</i>
	<i>Clone</i>
	<i>Origin: mouse</i>
	<i>Contains Natriumazide</i>
	<i>Titer</i>

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Instructions for use in other languages see:

<http://www.bag-diagnostics.com>

or phone +49 (0) 6404-925-125