

INSTRUCTIONS FOR USE

Anti-H monoclonal (IgM)



Clone: 10934C11

FOR IN VITRO DIAGNOSTIC USE

1. Product description

Anti-H monoclonal is prepared from monoclonal mouse IgM antibodies (Clone: 10934C11). The test reagent is designed for use in tube and plate tests. Anti-H is used for the determination of A-subgroups, since it reacts with erythrocytes of blood group A₂ and weak A-variants and reacts only weakly or not with erythrocytes of blood group A₁.

For stabilization the diluent used for this reagent contains bovine albumine and macromolecular substances. The test reagent contains < 0.1% NaN₃ as preservative.

The reactivity of each lot Anti-H monoclonal is demonstrated by all given methods with several red blood cells of blood group A₂ and 0. The titre given on the label is determined by the tube test method with reagent red blood cells and fresh red blood cells of blood group A₂.

2. Biological principle of the test

The test used 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 if the corresponding H substance is present in sufficient quantities on the test cells. Visible detection of this reaction is demonstrated by agglutination of the cells.

3. Storage and Shelf Life

Store Anti-H monoclonal at 2...8°C. Allow Anti-H monoclonal to reach room temperature (18...25°C) before use. Return reagent to 2...8°C for storage as appropriate immediately after use. After opening the bottle Anti-H monoclonal can be used until the expiration date printed on the label, if appropriate storage conditions be observed. Do not use the reagent after the expiry date printed on the label.

4. Specimen preparation

Blood samples should be collected by approved medical procedure. Blood collected without or with anticoagulant (heparin, citrate) is acceptable. Do not use haemolytic samples. Testing should be performed without delay if possible. Prolonged storage of red cells prior to testing may result in deterioration of red cell antigens and resultant false reactions (s. 9. Important Directions/Limitations of Procedure)

5. Additional Materials Required

Isotonic saline
Test plates for Blood Group Typing
Test tubes (75 x 12 mm)
Disposable Pasteur Pipettes
Centrifuge

6. Test procedure

Plate test

1. Prepare a 10% suspension of red cells to be tested in isotonic saline.
2. Place 1 drop of test reagent and 1 drop of the prepared suspension of red cells on a test plate and mix.
3. Incubate for 10 - 15 minutes at room temperature.
4. By slow rotation of the plate, examine macroscopically for agglutination.

Tube test

1. Prepare a ~ 3% suspension of red cells to be tested in isotonic saline.
2. Place 1 drop of test reagent and 1 drop of the prepared suspension of red cells into a labelled test tube and mix.
3. Incubate for 1 minute 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: Do not examine tests microscopically.

Red blood cell suspensions known to be A₁ positive and known to be A₂ 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 with tests with an Anti-A₁ test reagent and a test reagent without antibodies (negative control for monoclonal test reagents). By use of 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 or only weak agglutination with the Anti-A₁ test reagent refer to the phenotype A₂ 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-A₁ test reagent refer to the phenotype A₁.

If no strong agglutination occurs with the test red cells known to be A₂ positive or if a strong agglutination occurs with the test red cells known to be A₁ positive or the patient control and/or the test reagent without antibodies show a positive reaction the test results should not be interpreted.

If discrepant test results occurs with different test reagents, repeat the determination of the A subgroups with another test method and/or an other test reagent. Check doubtful results in plate test always in tube test. By problems of interpretation a titration of the test reagent may be helpful.

If testing with Anti-H test reagents, consider that red blood cells of blood group 0 carry the most H substance and react with Anti-H with the highest strength. In descend sequence follow weak A variants >A₂>A₂B>B>A₁>A₁B. For this reason red blood cells of blood groups B, A₁ and A₁B could also react with Anti-H. In case of A₂B, A₂ is attenuated by the neighbourhood of B. Thus cause weaker reactions with Anti-H test reagents. The very rare Bombay phenotype carry 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 procedure).

8. Stability Of The Reaction

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

9. Important directions / Limitations of Procedure

1. Anti-H monoclonal (IgM) is designed for in vitro diagnostic use only and should be used by properly trained individuals.
2. The determination of A subtypes with this test procedure is not possible in neonates since these subtypes are not yet sufficiently developed.
3. On rare occasion, red cells coated in vivo with immunoglobulin may agglutinate spontaneously and non-specifically. In such instances similar phenomena would most likely occur in the Rh grouping tests and blood grouping tests of other blood group systems as well. A patient autologous serum and a test reagent without antibodies are suitable controls. If the control tests yields a positive reaction, a valid interpretation of the A subgroup typing result cannot be made.
4. The use of unwashed test red cells suspended in plasma or serum may promote false positive reactions such as those associated with rouleaux formation, or autoantibodies. The use of well washed red cells may reduce the incidence of such false positive reactions.
5. Delays in reading tests, overvigorous resuspension of red cell buttons, and other technique variables associated with test performance may result in false test results.
6. Anti-H monoclonal (IgM) must not be used for tests with enzyme treated red cells.

7. Furthermore, to minimize other risks for false reactions, this reagent must not be tested when cold. Ensure that this reagent and any test cell sample are allowed to equilibrate to ambient room temperature prior to testing.
8. False reactions may occur with red cells that have been subjected to prolonged and/or inappropriate storage conditions.
9. Other variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware, incorrect saline pH and/or contaminated materials and samples may cause false negative or false positive results.
10. Microbiological contamination of Anti-H monoclonal (IgM) must be avoided as this may reduce the life of the product and cause erroneous results. Do not use Anti-H monoclonal (IgM) if marked turbidity or other observable indications of product alteration occur. These signs may indicate microbiological contamination and/or product deterioration.
11. 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 achieve the desired results.
12. For interpretation of the test results, consider if transfusion or transplantation had happened. Take the case history of the transfusion or transplantation and also the patient's medication history into consideration.

10. Performance characteristics

Samples of blood donors were tested in plate test (251 samples) and tube test (155 samples) with BAG-Anti-H, clone 10934C11. For verification all samples were tested also with another Anti-H test reagent (clone 107) and with an Anti-A₁ test reagent.

BAG-Anti-H, clone 10934C11, demonstrated stable agglutinations with samples of blood groups A₂, A₂B and O. As expected the agglutination in tube test was stronger than in plate test. With samples of blood groups A₁, A₁B and B BAG-Anti-H, clone 10934C11, showed no or in some cases weak agglutination. These agglutinations were not stable and could be resuspended easily. All test results could be verified with Anti-H test reagent, clone 107, and with the Anti-A₁ test reagent.

Tested samples (blood donors)	251
by that:	
Blood groups A ₂ , A ₂ B, O	124
Blood groups A ₁ , A ₁ B, B	127
Heparin blood	96
Citrat blood	155

11. 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 NaN₃ as a preservative. The reagent contains < 0.1% NaN₃ 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 specimen and test materials should be in accordance with state and local law.

12. Package: s. price list

13. Bibliography

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

Technical manual of the American Association of Blood Banks, 15th ed., 2005

Explanation of symbols used on Labelling	
	For in vitro diagnostic use
	Storage temperature
	Batch code
	Use by
REF	Catalogue number
	Consult instructions for use
	Monoclonal IgM
	Clone
	Origin: mouse
	Contains Natriumazide
	Titer

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