

US

MLB 2

Modified LISS Solution

FOR IN-VITRO DIAGNOSTIC USE

For tube test and TANGO[®] optimo

Package size

REF 805200100 VOL 50 mL MLB 2

Intended Use

MLB 2 is used for the TANGO[®] optimo, as a potentiator for red blood cells applied in the indirect antiglobulin (IAT) test for crossmatch, antibody screening and -identification, direct antiglobulin test (DAT) and for the determination of weak D and partial D antigens (DVI and DVII) with the Solidscreen II solid phase antiglobulin test.

MLB 2 is used for the tube test as potentiator for red blood cells applied in the indirect antiglobulin test (IAT) for crossmatch, antibody screening and -identification, as well as antigen typing with coombsreactive Blood Grouping Reagents.

Summary

In 1964 Mollison and Polley discovered that by reducing ion strength with a Low Ionic Strength Solution (LISS) antigen-antibody-reaction is considerably accelerated on blood group serological tests. The original formulation has been modified in MLB 2 so that an improved sensitivity is achieved.

MLB 2 is a modified LISS solution used as suspension medium for red cells and contributing to reaction time reduction while at the same time increasing reaction strength. In antibody determination with coombsreactive antisera, MLB 2 can be used in addition to the incubation medium.

Low Ionic Strength Salt Solutions (LISS) increase the rate of antigen-antibody complex formation.¹ Additionally, since antibody uptake is increased, incubation times of antigen-antibody reactions can be shortened. Excessive amounts of ions can interfere with binding of antibody to antigen.² Enhancement is achieved by decreasing the amount of ions in solution.

Principle

TANGO[®] optimo

Red blood cells are suspended in MLB 2/Alsevers Solution for cross match, DAT, weak D typing and in MLB 2 for antibody screening, -identification to be tested in the solid phase antiglobulin test Solidscreen II (please also refer to instructions for use of Solidscreen II).

Solidscreen II is a solid phase assay for

- the detection of red blood cell alloantibodies or autoantibodies in human plasma or serum.
- the determination of weak D and partial D antigens (DVI and DVII) of donor samples which have tested negative with IgM anti-D using Erytype S and the TANGO[®] optimo.

The Solidscreen II well is coated with Protein A. Protein A is a component of the cell wall of *Staphylococcus aureus* and has a very high affinity for the Fc portion of most immunoglobulin classes.³

For a) The plasma or serum and red blood cells (RRBC, donor or patient red blood cells) are added to the Protein-A coated well. Sensitization of the red cell occurs if the corresponding antibody is present for the antigen on the red cell.

For b) Solidscreen II Anti-D Blend and donor red blood cells are added to the Protein-A coated well. Sensitization of the red blood cell occurs if D antigen is present on the red blood cell.

Following incubation, and two wash processes to remove unbound protein, Anti-Human Globulin Anti-IgG Solidscreen II is added to the well. Following centrifugation, the well is evaluated. A smooth monolayer of cells is indicative of a positive reaction. A compact button of cells in the middle of the well is indicative of a negative reaction.

Tube test

The test principle is a hemagglutination test. MLB 2 reduces the incubation time in an indirect antiglobulin-test from 60 to 10 minutes while at the same time increasing reaction sensitivity

Reagent

MLB 2 is a low-ionic-strength solution containing phosphate buffer, glycine and bovine albumin. It is supplied in a 50mL glass bottle. The reagent is stable for seven days when opened and loaded on the TANGO[®] optimo. The reagent is stable until the expiration date when opened and used for the tube test.

- Preservative: 0.1% sodium azide
- No U.S. Standard of Potency
- For In-vitro Diagnostics use
- Use as furnished, do not dilute

Precautions

- For in vitro diagnostic use.
- Store at 2 to 8°C.
- Warning: Contains sodium azide, which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the build-up of explosive metal azides.**
- Caution: This product contains Natural Rubber Latex Which May Cause Allergic Reactions**
- Resuspend Reagent Red Blood Cells prior to be used and insert cell mixers before loading on TANGO[®] optimo
- Do not use if markedly turbid. Turbidity may be an indication of reagent contamination.
- Do not use past seven days on the TANGO[®] optimo.
- Do not use past the expiration date printed on the bottle.
- Do not use specimens collected with gel separators.

Specimen Collection

TANGO[®] optimo

For antibody screening, - identification (Indirect Antiglobulin Test IAT)

Fresh samples of clotted or EDTA anticoagulated whole blood can be used for antibody screening, - identification with the indirect antiglobulin test. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C. Use of samples older than seven days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used

There must be a distinct separation between the cellular and the plasma layer in the sample tube. Samples can be centrifuged or allowed to settle.

For crossmatch (Indirect Antiglobulin Test)

Fresh samples of EDTA or citrate anticoagulated whole blood samples must be used for the crossmatch. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C. Use of EDTA anticoagulated samples older than seven days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Donor blood stored in citrate anticoagulant at 1 to 6°C may be tested until the expiration date of the donor unit. These red blood cells to be tested must be prepared prior to testing. Refer to instructions in the TANGO[®] optimo Users Guide⁴. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used.

There must be a distinct separation between the cellular and the plasma layer in the sample tube. Samples can be centrifuged or allowed to settle.

For Direct Antiglobulin Test (DAT)

Fresh samples of EDTA anticoagulated whole blood samples or cord blood samples must be used for the Direct Antiglobulin Test. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, samples should be stored at 2 to 8°C. Use of samples older than seven days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used.

There must be a distinct separation between the cellular and the plasma layer in the sample tube. Samples can be centrifuged or allowed to settle.

For weak D and partial D antigen typing (Indirect Antiglobulin Test IAT)

Fresh samples of EDTA or citrate anticoagulated whole blood samples must be used for the weak D test. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed the EDTA anticoagulated samples should be stored at 2 to 8°C. EDTA anticoagulated whole blood samples may be tested for up to seven days following collection. Donor blood stored in citrate anticoagulant at 1 to 6°C may be tested until the expiration date of the donor unit. The red blood cells to be tested must be prepared prior to testing. Refer to instructions in the TANGO[®] optimo Users Guide. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used.

There must be a distinct separation between the cellular and the plasma layer in the sample tube. Samples can be centrifuged or allowed to settle.

Tube test

For antibody screening, - identification (Indirect Antiglobulin Test IAT)

Fresh samples of clotted or EDTA anticoagulated whole blood can be used for antibody screening, - identification with the indirect antiglobulin test. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA anticoagulated and clotted whole blood specimens should be stored at 2 to 8°C. Use of samples older than ten days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used

For crossmatch (Indirect Antiglobulin Test)

Fresh samples of clotted, EDTA or citrate anticoagulated whole blood samples must be used for the crossmatch. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA anticoagulated whole blood samples should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C. Use of samples older than ten days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples. Donor blood stored in citrate anticoagulant at 1 to 6°C may be tested until the expiration date of the donor unit. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used.

For antigen typing (Indirect Antiglobulin Test IAT)

Fresh samples of clotted or EDTA anticoagulated whole blood can be used for antigen typing with Blood Grouping Reagents for indirect antiglobulin test. Samples collected following standard blood sampling guidelines are acceptable. The specimen should be tested as soon as possible after collection. If testing is delayed, EDTA and clotted specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C. Use of samples older than ten days should be avoided unless there is no other alternative. Donor blood stored in citrate anticoagulant at 1 to 6°C may be tested until the expiration date of the donor unit. Stored samples should be allowed to reach room temperature prior to testing. Blood specimens exhibiting gross hemolysis or contamination should not be used.

Materials

Materials Provided

- MLB 2 in a 50mL glass bottle

Material required but not provided

TANGO[®] optimo

- TANGO[®] optimo REF 848900010
- Solidscreen II REF 806521100
- Biotestcell[®] 1 & 2 REF 816014100, Biotestcell[®] 3 REF 816085100, Biotestcell[®]-I 8 REF 816020100, Biotestcell[®]-I 11 REF 816021100, Biotestcell[®] Pool REF 816065100
- Alsevers Solution (Biotest REF 806510100)
- Search-Cyte[®] Pool, or Search-Cyte[®] Duo, or Search-Cyte[®] Trio for the TANGO[®]
- Anti-Human Globulin Anti-IgG Solidscreen II (Biotest REF 806516100)
- Solidscreen II Control (Biotest REF 806514100)
- Solidscreen II Control B (Biotest REF 806519100)
- Solidscreen II Negative control (Biotest REF 806509100)
- Phosphate Buffered Saline pH 7.3 ± 0.2
- Centrifuge
- Cell Mixers

Material required but not provided

Tube test

- Pipettes (drop volume 40 to 50 µL)
- Isotonic saline solution
- Biotestcell[®] 1 & 2 REF 816014100, Biotestcell[®] 3 REF 816085100, Biotestcell[®]-I 8 REF 816020100, Biotestcell[®]-I 11 REF 816021100)
- Donor or patient red blood cells
- Anti-Human Globulin Anti-IgG REF 804175100
- Anti-Human Globulin Anti-IgG, -C3d polyspecific REF 804115100

FOR REFERENCE USE ONLY: DO NOT USE in place of package inserts provided with each product.

- IgG coated red blood cells (e.g. Biotest Coombscell-E [REF] 816030100)
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological centrifuge
- Interval Timer
- Markers
- Isotonic Saline
- PBS pH 7.3 ± 0.2
- Optical aid (optional). The use of an optical aid for agglutination reading must be validated by the user.

Test procedure

TANGO[®] optimo

- MLB 2 is manufactured as ready to use for the TANGO[®] optimo.
- Please see the TANGO[®] optimo User Guide for loading instructions on the TANGO[®] optimo.

Tube test

A. Compatibility test

1. Prepare a 3 to 5 % suspension of donor red blood cells in isotonic saline solution.
2. In a properly marked tube mix 1 drop of red blood cell suspension and 2 drops recipient serum and 2 drops MLB 2.
3. Incubate for 10 minutes at 37°C.
4. Centrifuge for 20 seconds at 800 - 1000 x g.
5. Gently dislodge the cell button and observe for agglutination or hemolysis.
6. Wash red cells 3 times in isotonic saline solution. Completely decant supernatant.
7. Add Anti-Human Globulin according to manufacturers instructions and mix.
8. Centrifuge for 20 seconds at 800 - 1000 x g.
9. Gently dislodge the cell button and observe for agglutination.
10. Record results

Results

Positive cross match

= Agglutination and/or hemolysis

= Sensitization of donor red cells by antibodies or recipient serum.

Negative cross match

= No agglutination and/or hemolysis

= The recipient serum contains no antibodies against the donor red cells.

B. Antibody screening and antibody identification

1. In a properly marked tube mix 2 drops serum to be tested, 1 drop Reagent Red Blood Cells (3 to 5 %) and 2 drops MLB 2.
2. Incubate for 10 minutes at 37°C.
3. Centrifuge for 20 seconds at 800 - 1000 x g.
4. Gently dislodge the cell button and observe for agglutination or hemolysis.
5. Wash red blood cells 3 times in isotonic saline solution. Completely decant supernatant.
6. Add Anti-Human Globulin according to manufacturers instructions and mix.
7. Centrifuge for 20 seconds at 800 - 1000 x g.
8. Gently dislodge the cell button and observe for agglutination.
9. Record results

C. Antigen typing

The LISS antiglobulin-test reduces the time needed for antigen determination.

1. Prepare a 3 to 5 % suspension of red blood cells in isotonic saline solution.
2. In a properly marked tube mix the reagent (follow the directions of the Blood Grouping Reagent manufacturer), 1 drop red cell suspension and 2 drops MLB 2.
3. Incubate for 10 minutes at 37°C.
4. Wash red cells 3 times in isotonic saline solution. Completely decant supernatant.
5. Add Anti-Human Globulin according to manufacturers instructions and mix.
6. Mix and centrifuge for 20 seconds at 800 - 1000 x g.
7. Gently dislodge the cell button and observe for agglutination.
8. Record results

A positive and a negative control should be performed in parallel.

Stability of the Reaction

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

Quality Control

TANGO[®] optimo

A series of quality control samples must be run each day before testing or according to local requirements to ensure that the reagents, antisera and TANGO[®] optimo are functioning properly.

Controls should be run whenever:

- Lot numbers change (plate, reagent).
- A new bottle or preparation is placed on the system (red blood cells, AHG, MLB 2).
- After service/repair of the analyzer.

Two positive controls, Solidscreen II Control and Solidscreen II Control B are available for testing on the TANGO[®] optimo. The Solidscreen II Control contains diluted anti-D and Solidscreen II Control B contains diluted anti-c.

A negative control, Solidscreen II Negative Control is available for testing on the TANGO[®] optimo.

A minimum of one positive and one negative plasma/serum should be run for the Solidscreen II assay. The Solidscreen II Control can be used as the positive control.

Tube Test

A series of quality control samples must be run each day before testing or according to local requirements to ensure that the reagents, antisera and equipment are functioning properly.

A minimum of one positive and one negative plasma/serum should be run for the indirect antiglobulin test.

Interpretation

The tests are considered valid if the expected results for the controls are obtained. If the controls do not give the expected results, you must determine the cause for the failed QC.

Follow institutional SOP for repeat testing of QC samples, repeat testing of

187734/07
Rev. 01/2009

patient/donor samples and documentation of QC results and corrective action if required.

Interpretation of results

TANGO[®] optimo

The results are read immediately and a digital image is stored for review by the operator. Image algorithms contained in the TANGO[®] optimo evaluate, and provide an interpretation (positive or negative) for the well. The operator performs validation of the final results.

Positive Result: A layer of red blood cells across the bottom of the well.

Negative Result: A compact red blood cell button at the center of the well

Tube test

Agglutination of the red blood cells with the indirect antiglobulin test is a positive result and indicates the presence of an unexpected antibody(ies). Agglutination of the red blood cells with the direct antiglobulin test is a positive result and indicates an auto-agglutinin or auto antibodies. No agglutination is a negative result and indicates the absence of an unexpected antibody or the absence of the corresponding antigen or lack of an auto-agglutinin.

An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual, 15th edition).

Limitations

- Many antigen-antibody reactions are enhanced by Low Ionic Strength Solutions. However not all antibodies react optimally in a LISS test system (e.g. some IgM antibodies)
- The ionic strength of a test system is dependent on the amount of serum/plasma used. The addition of excess serum/plasma will increase the ionic strength and decrease the sensitivity of the test system.
- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin.
- If there is no anti-complement activity in the Anti-Human Globulin used, red blood cells coated with complement will not give a positive reaction.
- False test results may occur, but are not limited to:
 1. Contamination of sample or reagents
 2. Autoantibodies
 3. Improper storage or preparation of cells
 4. Incorrect incubation
 5. Incorrect calibration / centrifugation
 6. Incorrect reading technique
 7. Antibodies to antibiotics or other reagents in the TANGO[®] optimo Test System
 8. Cold Antibodies
 9. Screen cells not being mixed prior to loading on the TANGO[®] optimo (Please see **Precautions** section in this package insert regarding preparation of Reagent Red Blood Cells for TANGO[®] optimo).
- Positive reactions may be seen from individuals who have received Rh Immunoglobulin.
- Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.

Specific Performance Characteristics

The final release testing is performed according to the product specific SOPs. Each lot of Biotest MLB 2 reagent is tested in the Quality control by package insert method to insure suitable reactivity.

When properly stored and used according to the procedure described in the TANGO[®] optimo User Guide, MLB 2 may increase the rate of association between antibodies in human plasma/serum and red blood cell antigens.

For the product performance it is necessary to adhere to the recommended method in the instructions for use.

For Technical Support or further product information, contact Biotest Diagnostics Corporation at 800-522-0090.

Note

Each facility should verify the optimum spin time for the specific centrifuge in use. Manual techniques are to be performed according to the manufacturer's instructions. Each deviation from these instructions is the sole responsibility of the user. Used tests must be discarded as hazardous material. Manage waste according to national regulations.

Glossary of Symbols

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	In vitro diagnostic medical device
⚠	Caution, consult accompanying documents	📖	Consult instructions for use
🏭	Manufacturer	🕒	Use by YYYY-MM-DD
⚖	Contains sufficient quantity for <n> tests.	[REF]	Catalog number
🌡	Temperature limitation		

Bibliography

1. Issitt, Peter D., and Issitt, Charla H. Applied Blood Group Serology Oxnard, Calif.: Spectra Biologicals, 1979
2. Walsh, R.J. "The effect of electrolytes on the Rh agglutination reaction." Med J Aust 1948;i:793.
3. KJ Reis et al. Journal of Immunology 1984
4. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005

Key: Underline = Addition of changes ◀ = Deletion of text