US

Anti-Human Globulin

Anti-IgG Solidscreen II

(Rabbit)

FOR IN-VITRO DIAGNOSTIC USE
For Solidscreen II with the TANGO® optimo
U.S. License Number: 1798

Package size
REF 806516100 VOL 55 mL Anti-Human Globulin Anti-IgG Solidscreen II

Intended Use
Anti-Human Globulin Anti-IgG Solidscreen II for the TANGO® optimo is used for the indirect antiglobulin test to demonstrate the in-vitro IgG coating of red blood cells with antibody molecules as in antibody screening, antibody identification as well as crossmatch tests and for the use of the Solidscreen II Anti-D Blend Blood Grouping Reagent for weak D and partial D (DVI and DVII) antigen typing (with the indirect antiglobulin test).
Furthermore Anti-Human Globulin Anti-IgG Solidscreen II for the TANGO® optimo is used for the direct antiglobulin test to demonstrate the in-vivo coating of red blood cells with antibody molecules (such as autoantibodies, maternal antibodies in hemolytic disease of the newborn and stillbirth, alloantibodies against red blood cells in transfusion reactions).

Summary
Moreschi first described the use of Anti-Human Globulin in 1908[1]. Coombs rediscovered the test in 1945.[2,3] By injecting rabbits with human IgG, they were able to produce a protein (Anti-IgG) that reacted with incomplete antibodies (IgG). Most "incomplete" antibodies (IgG) fail to agglutinate red blood cells suspended in saline.[4] Most clinically significant antibodies in red blood cell serology are of the IgG class and can only be detected by the use of Anti-IgG. A stable lattice structure is formed and agglutination occurs when Anti-IgG binds to the IgG sensitized red blood cells.
Biotest Anti-IgG Solidscreen II reagent is used to test for the presence or absence of unexpected red blood cell antibodies. Furthermore, blood group weak D and partial D (DVI and DVII) antigen typing (with the corresponding test reagent for the indirect antiglobulin test) can be carried out. Routine pretransfusion studies always include tests for antibody screening, crossmatch and antibody identification.

Principle of the Test
The test principle is a solid phase assay for
a) the detection of red blood cell alloantibodies or autoantibodies in human plasma or serum.
b) The determination of weak D and partial D antigens (DVI and DVII) of 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.[5]
Sensitization of the red blood cell occurs if the corresponding antibody is present on the red blood cell. Following incubation, and two wash processes to remove unbound protein, Anti-Human Globulin is added to the well and acts as a link between the antibody coating of neighbouring red blood cells and induces solid phase. Uncoated red blood cells will form a red blood cell button. Following centrifugation, the well is evaluated. A smooth monolayer of red blood cells is indicative of a positive reaction. A compact button of cells in the middle of the well is indicative of a negative reaction.

Reagent
Anti-Human Globulin Anti-IgG Solidscreen II is prepared by immunizing rabbits with human IgG. The anti-IgG component contains antibody reactivity against light chain IgG and thus may also binds to IgA or IgM sensitized red blood cells. There is no activity with complement coated red blood cells.
The reagent is supplied in a 55 mL glass bottle.
Antibodies are diluted in a isotonic saline solution containing bovine albumin and as colorant Patent Blue and Tartrazin.

Anti-Human Globulin Anti-IgG Solidscreen II (Rabbit)

Preservative: 0.1% sodium azide.

Precautions
- For in vitro diagnostic use
- Resuspend Reagent Red Blood Cells prior to use and insert red blood cell mixers before loading on TANGO® optimo.
- Store between 2 to 8°C.
- Do not use if turbid.
- Handle and dispose of reagents as potentially infectious
- Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
- Caution: This product contains Natural Rubber Latex Which May Cause Allergic Reactions.
- **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.**
- Do not dilute.
- Do not use beyond the expiration date.
- Do not use beyond seven days when opened and loaded on the TANGO® optimo.
- Do not freeze.
- Do not use samples collected in gel separator tubes.
- The bovine albumin used for the production of this reagent is purchased from BSE-free US sources, Boval Company L.P. in Cleburne, Tx, USA and Millipore in Kankakee, IL, USA.

Specimen Collection
TANGO® optimo
For antibody detection and 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 seven days should be avoided 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 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 Guide [6]. 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, EDTA anticoagulated whole blood samples should be stored at 2 to 8°C. Use of samples older than seven days should be avoided 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. [4] 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.

Materials
Materials Supplied
- Anti-Human Globulin Anti-IgG Solidscreen II

Material required but not provided
- TANGO® optimo REF 848900010
- Solidscreen II microplates REF 806521100
- Biotestcell® Pool REF 816065100, 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
- MLB2 (Modified LISS Biotest) REF 805200100
- Solidscreen II Anti-D Blend REF 806530100
- Solidscreen II Control REF 806514100
- Solidscreen II Control B REF 806519100
- Solidscreen II Negative Control REF 806509100
- Alsevers Solution REF 806510100
- Centrifuge
- Isotonic Saline
- PBS pH 7.3 ± 0.2
- Cell mixers

Test Procedure

Indirect Antiglobulin Test (IAT)

1. TANGO® optimo dispenses 50µL of patient serum/plasma or control reagents into the Solidscreen II microplate well.
2. TANGO® optimo prepares an approx 1% suspension of Reagent Red Blood Cells with MLB 2. An approx 1% suspension of donor red blood cells (crossmatch) is prepared with MLB 2 and Alsevers Solution.
3. TANGO® optimo dispenses 50µL of the Reagent Red Blood Cells cells or donor red blood cells prepared in (2.) into the well with patient serum/plasma or control reagents.
4. The mixture is incubated for 20 minutes at 37°C.
5. The mixture is centrifuged following incubation.
6. The supernatant is aspirated and the strip is washed twice. Centrifugation follows each wash process.
7. 100µL of Anti-Human Globulin Anti-IgG Solidscreen II is added to the well and mixed.
8. Centrifugation by TANGO® optimo
9. Reaction is evaluated and interpreted by TANGO® optimo.

Direct Antiglobulin Test (DAT)

1. TANGO® optimo prepares an approx 1% suspension of patient or donor red blood cells with MLB 2 and Alsevers Solution.
2. TANGO® optimo dispenses 50µL of the patient red blood cells prepared in (1.) into the Solidscreen II microplate well.
3. Following centrifugation, the supernatant is aspirated and the strip is washed twice. Centrifugation follows each wash process.
4. 100µL of Anti-Human Globulin Anti-IgG Solidscreen II is added to the well and mixed.
5. Centrifugation by TANGO® optimo
6. Reaction is evaluated and interpreted by TANGO® optimo.

Weak D and partial D antigen (DVI and DVII) testing

1. The TANGO® optimo dispenses 50 µL of Solidscreen II Anti-D Blend Blood Grouping Reagent into the Solidscreen II well.
2. TANGO® optimo prepares a 1% suspension of donor red blood cells with MLB 2 and Alsevers Solution.
3. TANGO® optimo dispenses 50 µL of the donor red blood cells prepared in (2.) into the well with Solidscreen II Anti-D Blend Blood Grouping Reagent.
4. The TANGO® optimo mixes the serum/plasma or reagent and red blood cells.
5. The mixture is incubated for 20 minutes at 37°C.
6. The mixture is centrifuged following incubation.
7. The supernatant is aspirated and the strip (wells) is washed twice. Centrifugation follows each wash process.
8. 100 µL of Anti-Human Globulin Anti-IgG Solidscreen II is added to each well and mixed.
9. Centrifugation by TANGO® optimo

Reaction is evaluated and interpreted by TANGO® optimo

Stability of the Reaction

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 verification of the final results.

Quality Control

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 analyzer are functioning properly.
Controls should be run whenever:

- Lot numbers change (plate, reagent).
- A new bottle or preparation is placed on the system (Reagent Red Blood Cells, Anti-Human Globulin, Anti-IgG Solidscreen II, MLB 2).
- After service/repair of the TANGO® optimo

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 control should be run for the Solidscreen II assay. The Solidscreen II Control and Solidscreen II Control B can be used as the positive control and Solidscreen II Negative Control can be used as the negative control.

Interpretation of QC

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

Interpretation of Results

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 verification 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.

Limitations

- Low frequency antigens may not always be present on Reagent Red Blood cells and a double dose of antigen may be required to detect very weakly reacting antibodies. Therefore, negative reactions with the screening cells do not always indicate the absence of unexpected antibodies.
- 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 Anti-IgG Solidscreen II.
- There is no anti-complement activity with this product. Red blood cells coated with complement should not give a positive reaction.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold Antibodies
  - Reagent Red Blood 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.
- **Do not use frozen/deglycerolized and enzyme treated red blood cells**

Specific Performance Characteristics

Testing is performed in accordance with FDA recommended methods.
The final release testing is performed according to the product specific SOPs. Each lot of Biotest Anti-Human Globulin Anti-IgG Solidscreen II reagent is tested in the Quality control by package insert method against IgG red blood cell antibodies to insure suitable reactivity. In addition the reactivity of the reagent is confirmed with IgG coated red blood cells. The products meet FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs.

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

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	VOL	Volume

Bibliography

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4. Pittiglio, D. Harmening. Modern Blood Banking and Transfusion Practices. Philadelphia, PA: F.A. Davis, 1983.
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187733/09
Rev. 12/2008

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

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