

Blood Grouping Reagent

Anti-D (RH1) Blend

Solidscreen II Human Monoclonal Blend (BS221/H41 11B7)

FOR IN-VITRO DIAGNOSTIC USE

For Solidscreen II with the TANGO[®] optimo

MEETS FDA POTENCY REQUIREMENTS

U.S. License Number: 1798

Package size

REF 806530100 **VOL** 5 mL Solidscreen II Anti-D (RH1) Blend

Intended Use

Solidscreen II Anti-D (RH1) Blend is used with Solidscreen II and the TANGO[®] optimo for the detection of weak D and partial D antigens (DVI and DVII) except Rh33. It is used to test donor samples which have been tested negative with IgM anti-D using Erytype S in the TANGO[®] optimo.

Summary

The D (RH1) antigen is the most important red blood cell antigen after A and B. Cells that have the D (RH1) antigen are "Rh positive". Cells that do not have the D (RH1) antigen are "Rh negative". Soon after the discovery of the Rhesus factor, it became obvious that some red blood cells were weaker reacting with anti-D than other "normal" D-positive red blood cells (Stratton, 1946). These Rhesus antigens were grouped under the heading of Du. It was also apparent that some Du red blood cells reacted more strongly with anti-D reagents than others. The discovery of an allo-anti-D antibody in the serum of a D-positive donor was the first indication that the D antigen may consist - in mosaic fashion - of several different sub-units (epitopes). The Rh(D) characteristic of the red blood cells of such persons is described as "partial D". These -rare- variants have been classified into the categories DII thru DVII, depending on their reactivity with allo-anti-D and monoclonal antibodies.

On the basis of a host of new scientific findings, especially molecular genetic typing the weak expressions of D, originally described as Du, can now be placed into two groups: category DII thru DVII or Dweak Type 1, 2, 3 etc.

Since 30% to 85% of D negative people who receive a D positive transfusion develop anti-D², recipients and donors are routinely tested for this antigen. Some D positive red blood cells require incubation with an anti-D reagent and/or addition of Anti-Human Globulin for agglutination to occur.

Solidscreen II Anti-D (RH1) Blend Blood Grouping Reagent is used to test for the presence or absence of the weak D antigen of samples which have been tested negative with IgM anti-D using Erytype S in the TANGO[®] optimo.

Routine pretransfusion studies always include tests for the D antigen. Other Rhesus reagents like Biotest Anti-C (RH2), Anti- \bar{c} (RH4), Anti-E (RH3) and Anti-e (RH5) are used principally in the resolution of antibody problems or in family studies.

The ethnic origin influences the genotype, which can be seen in the table.

Incidence of the More Common Genotypes in D+ Persons¹

Antigens Present	Genotype		Incidence (%)	
	DCE	Mod. Rh-hr	Whites	Blacks
D,C,c,e	DCe/ce	R ₁ r	31.1	8.8
	DCe/Dce	R ₁ R ₀	3.4	15.0
	Dce/Ce	R ₀ r'	0.2	1.8
D,C,e	DCe/DcCe	R ₂ R ₁	17.6	2.9
	DCe/Ce	R ₁ r'	1.7	0.7
D,c,E,e	DcE/ce	R ₂ r	10.4	5.7
	DcE/Dce	R ₂ R ₀	1.1	9.7
D,c,E	DcE/DcE	R ₂ R ₂	2.0	1.3
	DcE/cE	R ₂ r'	0.3	<0.1
D,C,c,E,e	DCe/DcE	R ₁ R ₂	11.8	3.7
	DCe/cE	R ₁ r'	0.8	<0.1
	DcE/Ce	R ₂ r'	0.6	0.4
D,c,e	Dce/ce	R ₀ r	3.0	22.9
	Dce/Dce	R ₀ R ₀	0.2	19.4

Principle of the Test

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 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.² Solidscreen II Anti-D (RH1) Blend and red blood cells to be tested 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, IgG Solidscreen II is added to the well. 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

As the reactive components Solidscreen II Anti-D (RH1) Blend contains human monoclonal antibodies of the immunoglobulin classes IgG and therefore is suited for testing with Solidscreen II. The antibodies are derived from cell culture supernatant and demonstrate the consistent specificity and reproducibility characteristic for monoclonal antibodies. The antibodies are diluted in AB serum containing bovine albumine.

Solidscreen II Anti-D (RH1) Blend (clones: BS221/ H41 11B7 (IgG/IgG))

Preservative: 0.1% sodium azide.

Precautions

- For In-vitro diagnostic use.
- Store at 2 to 8°C.
- Do not use beyond the expiration date.
- Do not use if turbid.
- Handle and dispose of reagents as potentially infectious
- Caution: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED WITH FDA LICENSED EIA/ELISA TESTS. NAT TESTING WAS NOT PERFORMED. NO KNOWN TEST METHOD CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS
- 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 (NaN₃), 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.
- 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

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 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.

Materials

Materials Provided

- Solidscreen II Anti-D (RH1) Blend

Materials and Equipment required but not provided

- TANGO[®] optimo [REF] 848900010
- Solidscreen II microplates [REF] 806521100
- MLB 2 (Modified LISS Biotest) [REF] 8065200100
- Alsevers Solution [REF] 806510100
- Donor or patient red blood cells
- Anti-Human Globulin Anti-IgG Solidscreen II [REF] 806516100
- Phosphate Buffered Saline pH 7.3 ± 0.2
- Cell Mixers

Stability of the Reactions

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

Test Procedure

TANGO[®] optimo

Detailed test procedure instructions as well as details for the evaluation of test results are given in the instructions for use of Solidscreen II microplates and TANGO[®] optimo User's Guide.

Quality Control

A series of positive and negative quality control samples must be run each day before testing to ensure that the reagents and analyzer are functioning properly.

To confirm the reactivity or specificity of Biotest Monoclonal Rh Blood Grouping Reagent Anti-D (RH1) for Solidscreen II, it should be tested with weakened antigen-positive and antigen-negative red blood cells, respectively. The reagent is satisfactory for use if it reacts only with antigen-positive red blood cells.

Controls also should be run whenever:

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

Interpretation of results

In a positive result, a stable lattice structure is formed and is seen as a layer of red blood cells across the bottom of the well.

A negative result is seen as a compact red blood cell button at the center of the well, as no lattice has been formed.

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

Reagent sera with donor red blood cells				Interpretation
Anti-D	Control	D ^{weak} -Test	DAT**	
+	0	/	/	Rh positive
0	0	0	0	Rh negative
0	0	+	0	* Rh positive
0	0	+	+	Invalid Test
+	+	/	/	Invalid Test

+ = positive

0 = negative

* A test for weak D may be performed on samples that test negative with Anti-D to determine the Rh status. A reagent containing an IgG Anti-D must be used. Certain groups of patients may require testing for weak D. Follow facility specific policies guidance for determining which samples require weak D testing.

**Testing is not valid unless the sample can be shown to react negatively with an appropriate Rh control or exhibits a negative direct antiglobulin test.

Frequencies in the population are listed in the "Summary" section.

187817/05
Rev. 07/2008

Limitations

- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-IgG Solidscreen II.
- 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 in the TANGO[®] optimo
 - Cold Antibodies

In case of questionable results of unknown origin contact Biotest (800-522-0090) for assistance.

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 Blood Grouping Reagent is tested in the Quality control by package insert method against a panel of antigen positive red blood cells (heterozygous antigen expression and if possible weakened antigen expression) to insure suitable reactivity. 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.

The Anti-D reagents have not been tested with rare phenotypes –D-, .D., Rhmod and Rhnull. The reactions with enzyme treated red blood cells has not been determined.

Biotest Solidscreen II Anti-D (RH1) Blend is a monoclonal blend of two IgG clones suitable for the Solidscreen II Antiglobulin test with the TANGO[®] optimo to determine weak D's except Rh33 of previously typed samples which have tested negative with IgM anti-D using Erytype S and the TANGO[®] optimo.

No blood grouping reagent of monoclonal origin has yet been found that will detect all parts of the D antigen

The performance of the Biotest Anti-D (RH1) Blend for Solidscreen II was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

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

Note

Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

Glossary of Symbols

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	<i>In vitro</i> 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

1. Mark E. Brecher, MD et al. Technical Manual 15th Edition, Bethesda, MA: AABB, 2005.
2. Reis AJ, Ayoub EM, Boyle MD. Journal of Immunology. 1984;132: 3091-3097