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® instruments
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1845

Package size
REF 806530100 VOL 5 mL Solidscreen™ II Anti-D (RH1) Blend

Intended Use
Solidscreen™ II Anti-D (RH1) Blend is intended for the detection of weak D and partial D antigens (DVI and DVII) except Rh3H with the solid phase assay Solidscreen™ II on TANGO® instruments (TANGO® optimo and TANGO infinity™). It is used to test blood samples that are negative when tested with IgM anti-D using Erytype® S.

Only donor samples are approved for testing on 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 Rh 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 RhD antigens were grouped under the heading of Dn. It was also apparent that some Dn 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 different sub-units (epitopes). The RhD antigen of the red blood cells of such persons is described as “partial D”. These -rare- variants have been classified into the categories DIII thru DVII, depending on their reactivity with allo-anti-D and monochlonal antibodies.

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

Since 30% to 85% of D negative people who receive a D positive transfusion develop anti-D', 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 or partial D antigen (DVI and DVII) of samples which are negative with IgM anti-D using Erytype® S.

Routine pretransfusion studies always include tests for the D antigen. Other Rh antigens were grouped under the heading of Dn. It was also apparent that some Dn red blood cells reacted more strongly with anti-D reagents than others.

Incidence of the More Common Genotypes in D+ Persons

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Incidence (%)</th>
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<tbody>
<tr>
<td>D,C,e</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/ce</td>
<td>R³ Rh-hr</td>
</tr>
<tr>
<td>D,C,e/Dce</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/Cce</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/Cde</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/Cdce</td>
<td>R² Rh-hr</td>
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<tr>
<td>D,C,e/Dcde</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De/C</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De/Cc</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De/Ce</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De/Cc</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De/Cc/D</td>
<td>R² Rh-hr</td>
</tr>
<tr>
<td>D,C,e/De/Cc/Dc</td>
<td>R² Rh-hr</td>
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</tbody>
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Principle of the Test
Solidscreen® II is a solid phase assay for:

- The detection of red blood cell antibodies 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.

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. 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-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 albumin.

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 past seven days on the TANGO® instruments.
- 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.
- Warning: Contains sodium azide (Na3N), 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 sourced from donor animals of U.S. origin that have been inspected and certified by U.S. Veterinary Service inspectors to be disease free.

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. Donor segments stored in citrate segments must be transferred to a secondary tube prior to testing on the instruments. A minimum volume of 500 µL of red blood cells is required in the secondary tube.

Donor and patient samples can be tested on TANGO infinity™. Testing of cord blood samples on TANGO infinity™ is only approved by Health Canada.

Only donor samples are approved for testing on the TANGO® optimo. 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 layers 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]
- TANGO infinity™ [REF 85000010]
- Solidscreen™ II microplates [REF 806521100]
- MLB 2 (Modified LISS Bio-Rad) [REF 80520010]

b) MEETS FDA POTENCY REQUIREMENTS
Test Procedure
Detailed test procedure instructions as well as details for the evaluation of test results are given in the respective TANGO instrument User Manual.

Quality Control
Quality control samples must be run each day before testing to ensure that the reagents and automated system components are functioning properly. Please refer to the instruments User Manual for recommended instrument quality control.

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

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 instrument 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.

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 components
  - Cold antibodies
- The performance characteristics have not been established with frozen/deglycerolised or enzyme treated cells.

In case of questionable results of unknown origin contact Bio-Rad Laboratories Inc. (800-224-6723) 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. As part of the lot release, each lot of Bio-Rad Blood Grouping Reagent is tested according to the 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 product meets FDA potency requirements.

The specificity 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 have not been determined.

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

No Blood Grouping Reagent of monoclonal origin has yet been found that will detect all parts of the D antigen.

The performance of the Bio-Rad Anti-D (RH1) Blend for Solidscreen II was confirmed against a FDA approved reference reagent in a multi-center clinical trial.

For Technical Support or further product information, contact Bio-Rad Laboratories Inc. at 800-224-6723.

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

Bibliography

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