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BLOOD GROUPING REAGENT
Anti-S (Monoclonal)  Anti-s (Monoclonal)
Gamma-clone®
By Tube and Microplate Tests

Preservative: <0.1% Sodium Azide 1°C to 10°C  Meets FDA Potency Requirements IVD

CAUTION: THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) MAY CONTAIN DRY NATURAL RUBBER. DO NOT PIPETTE THIS PRODUCT BY MOUTH, AS THE ABSENCE OF MURINE VIRUS HAS NOT BEEN DETERMINED.

DO NOT USE IF MARKEDLY TURBID.

Gamma-clone® Anti-S (Monoclonal) and Anti-s (Monoclonal) Blood Grouping Reagents are intended for the detection of the S (MNS3) and s (MNS4) antigens, respectively, on red blood cells by tube and microplate tests.

Summary of the Test:
The S and s antigens are inherited as if they are products of allelic genes.1,2 The locus at which S and s are situated is closely linked to the locus that carries M and N.3 Three different red blood cell phenotypes result from the inheritance of either or both of these genes (S+s+, S+s-, S-s+). They can be determined in serologic tests employing the appropriate antibodies. The red blood cells of a fourth phenotype, S-s-, fail to react with these sera altogether.4,5 Persons with this phenotype inherit genes that do not produce S or s. Some antibodies to S and s antigens may be naturally occurring, while others are formed in response to transfusion or pregnancy. Anti-S antibodies are nearly always immune in origin.6

Gamma-clone Anti-S (Monoclonal) and Gamma-clone Anti-s (Monoclonal) Blood Grouping Reagents are used to test patient or donor red blood cells for the presence of the S and s antigen, respectively. Typing of donor red blood cells facilitates the selection of suitable antigen-negative units for transfusion to patients with either of these antibodies. Red blood cell typing also serves as final verification of the identification of allo-anti-S or allo-anti-s in patient or donor samples.

Principle of the Test:
The presence of the S or s antigen is determined by testing with Anti-S or Anti-s, respectively, using tube or microplate test techniques. Agglutination of the test red blood cells constitutes a positive test result and indicates the presence of the relevant antigen. No agglutination constitutes a negative test result and indicates that the antigen is not present.

Reagents:
Gamma-clone Anti-S (Monoclonal) Blood Grouping Reagent is prepared from IgM antibodies from the human/murine heterohybridoma cell line MS-94 grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Sodium azide is added as a preservative (at less than 0.1% w/v). Ready for use as supplied.

Gamma-clone Anti-s (Monoclonal) Blood Grouping Reagent is prepared from IgM antibodies from the human/murine heterohybridoma cell line P3BER grown in fluid culture and suitably diluted in a proprietary diluent containing bovine albumin to achieve the appropriate level of potency for the test procedure as described. Sodium azide is added as a preservative (at less than 0.1% w/v). Ready for use as supplied.

Any Bovine Albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This ruminant-based product is deemed to have a low-TSE (Transmissible Spongiform Encephalopathy) risk.

Storage:
- Store at 1°C to 10°C when not in use.
- Do not use beyond the expiration date which is expressed as CCYY-MM-DD (year-month-date).
- Do not freeze.

Key:
Underline = Addition or significant change ▲ = Deletion of text
Test Method:

Automated Microplate Method:
For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Manual Tube Method:
1. Place one (1) drop of Gamma-clone Anti-S (Monoclonal) or Gamma-clone Anti- (Monoclonal) into a properly labeled test tube.
2. Add one (1) drop of an approximate 2-5% suspension of the red blood cells to be tested to the test tube (from step 1 above). The red blood cells to be tested should previously have been washed at least one time and resuspended in saline.
3. Mix the test tube contents well by gently shaking the tube and incubate the tube for five (5) to fifteen (15) minutes at room temperature (15ºC to 30º C). Incubating for the upper end of the time range may enhance reactivity.
4. Centrifuge the test tube.*
5. After centrifugation, immediately resuspend the red blood cells by gently shaking the test tube and examine for macroscopic agglutination. Negative reactions may be examined with an optical aid; however, microscopic reading is not recommended. Record the results.

*Suggested centrifugation time and RCF: 15 to 30 seconds at 900-1000 xg or a time and resulting phenotypes in some ethnic populations.

Limitations:
Factors that may cause false test results include the following:
- a. Bacterial or chemical contamination of blood specimens, reagent and/or supplementary materials.
- b. Improper storage of materials.
- c. Aged or stored blood specimens. Such specimens may yield weaker reactions than those obtained with fresh red blood cells.
- d. Too heavy a red blood cell suspension of the specimen.
- e. Improper incubation time or temperature.

Table 1: The reaction patterns of Anti-S and Anti- s are shown in Table 1, together with the frequencies of the resulting phenotypes in some ethnic populations.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Reagent</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-S</td>
<td>Anti-s</td>
</tr>
<tr>
<td>S+s+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S+s-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S-s+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>S-s-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Interpretation of Results:

Automated Microplate Method:
For the interpretation of results associated with automated instrumentation, refer to information provided in the instrument operator manual.

Manual Tube Method:
Agglutination of the red blood cells constitutes a positive test result and indicates the presence of the S or s antigen, dependent on the specific reagent used.

No agglutination constitutes a negative test result, and indicates the absence of the S or s antigen, dependent on the specific reagent used.

The reaction patterns possible with Anti-S and Anti- s are shown in Table 1, together with the frequencies of the resulting phenotypes in some ethnic populations.

Stability of Reaction:
Following centrifugation, the test tube should be read immediately and interpreted without delay.

Automation reads and interprets the results immediately.

Quality Control:
The reactivity of blood grouping reagents should be confirmed on each day of use by testing with red blood cells known to be negative and positive for the relevant antigens. S+s+ red blood cells are the most suitable positive control red blood cells for Gamma-clone Anti-S (Monoclonal) and Gamma-clone Anti- (Monoclonal). Each reagent is satisfactory for use if it reacts only with antigen-positive red blood cells. Immucor Reagent Red Blood Cells are a convenient source of control cells and may be used as supplied.

Interpretation of Results:

Specific Performance Characteristics:
Gamma-clone Anti-S (Monoclonal) and Anti- (Monoclonal) meets FDA potency requirements. Each lot is tested by insert methods against a panel of antigen-positive and antigen-negative red blood cells to ensure suitable reactivity and specificity. The specificity of the murine monoclonal antibodies secreted by the cell lines used to manufacture these Blood Grouping Reagents has been determined by testing with red blood cells of varying phenotypes.

The performance of this product is dependent upon adhering to the package insert recommended methodology.

Performance Characteristics by manual tube method:
Method comparison studies were performed at three (3) external sites representative of blood collection establishments, hospital-based transfusion services, and clinical laboratories. Specimens were tested with the reagent and also a comparator reagent. Test results were evaluated for agreement between reagents. Combined results from all sites are summarized in the following tables:

Note: Agreement between methods does not indicate which method is correct.

<table>
<thead>
<tr>
<th>Results</th>
<th>Comparator Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-S</td>
<td>Positive Percent Agreement</td>
</tr>
<tr>
<td>Positive</td>
<td>550</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

Key:
- Underline = Addition or significant change
- ▲ = Deletion of text
Performance Characteristics on NEO Iris and Galileo NEO:

**CE-marked assays:** Method comparison studies were performed at one (1) internal site. Specimens were tested with the reagent and also a comparator reagent. Samples with initial equivocal results were retested. Test results were evaluated for agreement between reagents. Note: Agreement between methods does not indicate which method is correct.

<table>
<thead>
<tr>
<th>Results N=1030</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>940</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Anti-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>555</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>406</td>
<td></td>
</tr>
</tbody>
</table>

For CE-marked assay only; not applicable to North American Market.

<table>
<thead>
<tr>
<th>Results N=961</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>804</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-s</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
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<td>157</td>
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<table>
<thead>
<tr>
<th>Results N=975</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>545</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Anti-s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2**</td>
<td>426</td>
<td></td>
</tr>
</tbody>
</table>

Discordant samples were further genotyped by DNA molecular testing (PreciseType™ HEA BeadChip). *One (1) sample gave mixed-field reaction with comparator reagent; one (1) sample gave mixed-field reaction on NEO Iris. **One (1) sample gave mixed-field reaction with comparator reagent; one (1) sample resulted negative on NEO Iris and positive with comparator reagent, and was negative by HEA BeadChip.

<table>
<thead>
<tr>
<th>Results N=975</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>793</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Anti-s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>179</td>
<td></td>
</tr>
</tbody>
</table>

Discordant samples were further genotyped by DNA molecular testing (PreciseType™ HEA BeadChip). *Two (2) samples initially typed s– with comparator reagent; repeat test with comparator reagent and DNA was s+. One (1) sample initially typed s– with comparator reagent; repeat tests with comparator reagent and NEO Iris were s–; DNA was s+. Resolved NPA 99.44% (97.39% 95% 1-sided LCI). The Lower 99% CI was not met due to the lower number of s– samples in the population, N=179.

**Precision studies**
Repeatability and Reproducibility were performed at three (3) sites by testing identical sample panels, containing positive and negative panel members in triplicate, testing two runs per day for five non-consecutive days. Results demonstrated 100% agreement for all positive and negative panel members.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (855 466-6267).

EU/EEA/EFTA: Report any serious incident involving these reagents to Immucor, or the distributor, and the National Competent Authority of the Member State in which the user and/or patient is established. Summary of Safety and Performance can be found at w.immucor.com, select Customer Login, Customer Center.

**Bibliography:**


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