

**Anti-Human Globulin
Anti-IgG (Rabbit)**

**For Tube Technique**

**DOES NOT CONTAIN ANTIBODIES TO COMPLEMENT COMPONENTS**

- **FOR IN VITRO DIAGNOSTIC USE**
- **Meets FDA potency requirements**
- **Discard if turbid**
- **Preservative: 0.1% (w/v) sodium azide**

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**INTERPRETATION OF LABELING SYMBOLS**

- **LOT**
  - Batch code
- **REF**
  - Product code
- **PO**
  - Storage temperature limitation (2–8 °C)
- **IVD**
  - In vitro diagnostic medical device
- **Consult instructions for use**
- **Manufacturer**

**INTENDED USE**

Anti-Human Globulin, Anti-IgG, is intended for use in the direct antiglobulin test to detect the in vivo coating of human red blood cells with IgG.

Anti-Human Globulin, Anti-IgG is intended for use in the indirect antiglobulin test to detect the in vivo coating of human red blood cells with IgG.

**CAUTION: THE ABSENCE OF ALL VIRUSES HAS NOT BEEN DETERMINED. THIS PRODUCT HAS COMPONENTS (DROPPER BULBS) CONTAINING DRY NATURAL RUBBER**

**REFERENCES**

Z356U

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**MATERIALS**

- **Material provided**
  - Anti-Human Globulin Anti-IgG
- **Materials required but not provided**
  - Isotonic saline
  - Reagent red blood cells
  - Donor or patient red blood cells/serum
  - IgG sensitized red blood cells
  - 10 x 75 mm or 12 x 75 mm glass test tubes
  - Pipettes
  - Centrifuge
  - Timer

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**PROCEDURES**

**NOTE:** This reagent has been standardized for use by the techniques described below and therefore its suitability for use by other techniques cannot be guaranteed. When a test is required to be incubated for a specific time period, a timer should be used.

### Indirect Antiglobulin Test

If an enhancement medium/potentiator or a blood typing reagent is used, please refer to the manufacturer’s instructions for use.

- **Procedure:**
  - Prepare a 2-4% suspension of red blood cells in isotonic saline solution. (Reagent Red Blood Cells may be used directly from the vial or according to the manufacturer’s instructions).
  - Add 2 drops of the serum or plasma to be tested to a glass test tube.
  - Add 1 drop of red blood cell suspension. Steps 2 and 3 may be performed in either order.
  - Mix the contents of the test tube well and incubate at 37 °C ± 1 °C for 30-60 minutes or according to the manufacturer’s instructions if a potentiator is being used.
  - Wash the test 3-4 times with a large excess of isotonic saline. (e.g. 4 mL of saline per 10 (or 12) x 75 mm glass test tube.)

**NOTE:**
- (i) allow adequate spin time to sediment the red blood cells.
- (ii) make sure that the residual saline is removed at the end of each wash.
- 6. Add 2 drops of Anti-Human Globulin Anti-IgG to each test tube.
- 7. Mix the contents of the test tube well and centrifuge. **NOTE:** Suggested centrifugation: 900-1000 g (approx. 3400 rpm) for 10 seconds or a time and speed appropriate for the centrifuge used that produces the strongest reaction of positive tests, yet allows easy re-suspension of negative tests.
- 8. After centrifugation, gently shake the test tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination. Negative reactions may be examined with an optical aid.
- 9. Record results.
- 10. To all negative tests add IgG sensitized reagent red blood cells.
  - a) Add 1 drop of IgG sensitized reagent red blood cells to each negative anti-human globulin test.
  - b) Mix the contents of the test tube well and centrifuge. **NOTE:** Suggested centrifugation: 900-1000 g (approx. 3400 rpm) for 10 seconds or a time and speed appropriate for the centrifuge used that produces the strongest reaction of positive tests, yet allows easy re-suspension of negative tests.
  - c) After centrifugation, gently shake the test tube to dislodge the cell button from the bottom and immediately observe macroscopically for agglutination.
  - d) Any test which does not show a positive reaction should be considered invalid and repeated.

**STABILITY OF REACTION**

Test results should be read and interpreted immediately after centrifugation. Delays may cause dissociation of antigen-antibody complexes resulting in weak positive or false negative reactions.

**INTERPRETATION OF RESULTS**

Agglutination of the test red blood cells in either the direct or indirect antiglobulin test indicates a positive test result with detectable IgG present on the surface of the red blood cells.

No agglutination of the test red blood cells in either the direct or indirect antiglobulin test indicates a negative test result with no detectable IgG present on the surface of the red blood cells.

**QUALITY CONTROL**

Quality control of reagents is essential and should be performed daily. All negative antiglobulin tests should be controlled using IgG sensitized reagent red blood cells. A positive result indicates the presence of active anti-IgG. Tests in which negative results are obtained with this procedure should be considered invalid and repeated if necessary. Routine quality control should confirm that the anti-human globulin contains active anti-IgG. Anti-IgG reactivity can be checked by testing the Anti-Human Globulin reagent with IgG sensitized red blood cells.

Any reagent red blood cell with a negative direct antiglobulin test may be used as a negative control, if desired.

**LIMITATIONS**

**NOTE:** Any saline present after the completion of the wash phase may dilute the Anti-Human Globulin Anti-IgG reagent beyond its optimal working concentration. Therefore, it is important to ensure that the maximum amount of wash solution is removed after each centrifugation step.

Heating blocks and waterbaths promote better heat transfer and are recommended for 37 °C tests.

Gently re-suspend test tubes before reading. Excessive agitation may disrupt weak agglutination and produce false negative results.
Excessive centrifugation can lead to difficulty in re-suspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

SPECIFIC PERFORMANCE CHARACTERISTICS

Comparator Study Results

During comparator studies (data on file at Alba Bioscience Limited), blood samples were tested with Anti-Human Globulin Anti-IgG as follows:

Indirect Antiglobulin Test

<table>
<thead>
<tr>
<th>Anti-IgG</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>One-sided 95% Exact Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>2187</td>
<td>0</td>
<td>2187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>6610</td>
<td>6611</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2188</td>
<td>6610</td>
<td>8798</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive Percent Agreement* = 99.95 0.99

Negative Percent Agreement* = 100.00 0.99

* Indicates agreement between the Anti-Human Globulin Anti-IgG and comparator reagents only and does not indicate which reagent gave the correct result(s).

In performance evaluation studies, 8798 samples were tested with Anti-Human Globulin Anti-IgG. The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. The negative agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. Both the positive and negative percent agreement met the acceptance criteria of 0.99 at the one sided 95% lower confidence limit.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

- ABO Cross-Match

<table>
<thead>
<tr>
<th>Anti-IgG</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>One-sided 95% Exact Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>119</td>
<td>0</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>221</td>
<td>221</td>
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<td></td>
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<tr>
<td>Total</td>
<td>119</td>
<td>221</td>
<td>340</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive Percent Agreement* = 100.00 0.99

Negative Percent Agreement* = 100.00 0.99

* Indicates agreement between the Anti-Human Globulin Anti-IgG and comparator reagents only and does not indicate which reagent gave the correct result(s).

Of the 8798 IAT samples tested in performance evaluation studies with Anti-Human Globulin Anti-IgG, 340 samples were tested for ABO cross-match. The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. The negative percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. Both the positive and negative percent agreement met the acceptance criteria of 0.99 at the one sided 95% lower confidence limit.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

- Non-ABO Cross-Match

<table>
<thead>
<tr>
<th>Anti-IgG</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>One-sided 95% Exact Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>303</td>
<td>0</td>
<td>303</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>545</td>
<td>545</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>303</td>
<td>545</td>
<td>848</td>
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</tr>
</tbody>
</table>

Positive Percent Agreement* = 100.00 0.99

Negative Percent Agreement* = 100.00 0.99

* Indicates agreement between the Anti-Human Globulin Anti-IgG and comparator reagents only and does not indicate which reagent gave the correct result(s).

Of the 8798 IAT samples tested in performance evaluation studies with Anti-Human Globulin Anti-IgG, 488 samples were tested for non-ABO cross-match. The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. The negative percent agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. Both the positive and negative percent agreement met the acceptance criteria of 0.99 at the one sided 95% lower confidence limit.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

Direct Antiglobulin Test

<table>
<thead>
<tr>
<th>Anti-IgG, Anti-Clq</th>
<th>Comparator Reagent</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>One-sided 95% Exact Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>265</td>
<td>1</td>
<td>266</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>1253</td>
<td>1254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>1254</td>
<td>1520</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive Percent Agreement* = 99.62 0.98

Negative Percent Agreement* = 99.92 0.99

* Indicates agreement between the Anti-Human Globulin Anti-IgG and comparator reagents only and does not indicate which reagent gave the correct result(s).

In performance evaluation studies, 1520 samples were tested with Anti-Human Globulin Anti-IgG. The positive percent agreement at the one-sided 95% exact lower confidence limit was 0.98 for agglutination tests based on a comparison of interpreted results. The negative agreement at the one-sided 95% exact lower confidence limit was 0.99 for agglutination tests based on a comparison of interpreted results. The positive percent agreement did not meet the acceptance criteria of 0.99 at the one sided 95% lower confidence limit due to the low frequency of positive samples encountered and one discrepant result which was a sample from a patient historically presenting with a positive DAT.

Results were evaluated against comparable FDA approved products using the appropriate methods for the comparators.

Precision Study Results

As part of the performance evaluation, precision and lot to lot studies were performed using multiple operators, days and runs to confirm repeatability and reproducibility of test results in the same run, day and with the same operator and between runs, days and operators. The study took account of inter-lot variables such as days of the week, times of day and supplementary reagents used in testing.

There were no discordant results; all expected positive test outcomes generated unequivocal positive reactions and all expected negative test outcomes generated unequivocal negative reactions.

Prior to release, each lot of Anti-Human Globulin Anti-IgG is tested by FDA recommended methods against IgG and complement coated red blood cells to ensure suitable reactivity.

BIBLIOGRAPHY


DATE OF ISSUE

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