Anti-Human Globulin Anti-IgG

(Rabbit)
For Tube Technique

DOES NOT CONTAIN ANTIBODIES TO COMPLEMENT COMPONENTS

REF **Z356U**

REF **Z357U (Green)**

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

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

INTERPRETATION OF LABELING SYMBOLS

Batch code


Use by (YYYY-MM-DD)

Product code

Storage temperature limitation (2–8 °C)

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 vitro* coating of human red blood cells with IgG.

SUMMARY AND EXPLANATION

The antiglobulin test was first used in blood group serology by Coombs, Mourant and Race in 1945. The serum of animals immunized with human protein was used to detect 'incomplete' antibodies bound to red blood cells.

The direct antiglobulin test will detect IgG antibodies bound to red blood cells *in vivo* in serological conditions such as the presence of autoantibodies, antibodies as a result of a transfusion reaction and hemolytic disease of the fetus and newborn.

The indirect antiglobulin test will detect, after incubation of serum or plasma with red blood cells, IgG antibodies bound to red blood cells *in vitro* in applications including antigen typing, antibody detection, and antibody identification.

PRINCIPLE OF THE TEST

The Anti-Human Globulin Anti-IgG will cause agglutination of red blood cells sensitized with IgG. No agglutination will be observed with uncoated red blood cells.

REAGENT DESCRIPTION

The main component of this reagent is rabbit antibody to human IgG.
The formulation also contains bovine serum albumin, 0.1% (w/v) sodium azide and Tween 80. The Z357U reagent is dyed green by the addition of patent blue and tartrazine.
NOTE: The volume delivered by the reagent bottle dropper is approximately 40 µL. Care should be taken to ensure that appropriate serum to cell ratios are maintained in all test systems.

STORAGE

The reagent should be stored at 2–8 °C.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only.
Product should be used by qualified personnel.
Do not use beyond expiration date.
Do not use if turbid.
Do not dilute.
The format of the expiration date is expressed as YYYY-MM-DD (Year-Month-Day).

This reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide buildup.
This reagent is of animal origin, therefore care must be taken during use and disposal as there is a potential infection risk.
This product has components (dropper bulbs) containing dry natural rubber.

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected by a standard collection technique. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at refrigerated temperatures. Do not use blood specimens that exhibit contamination. Extreme care should be taken if hemolyzed samples must be tested. Clotted samples, or those collected in EDTA, should be tested within fourteen days from collection. Donor blood may be tested until the expiration date of the donation.

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
- Heating block/waterbath
- Optical aid (optional)
- Potentiator of choice (optional)
  - Bovine Serum Albumin
  - LISS Additive
  - PEG

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 respective instructions for use.
1. 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).
2. Add 2 drops of the serum or plasma to be tested to a glass test tube.
3. Add 1 drop of red blood cell suspension. Steps 2 and 3 may be performed in either order.
4. 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.
5. 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 immediately. 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 red blood cells and follow manufacturer's instructions. Any test which does not show a positive reaction should be considered invalid and repeated.

Direct Antiglobulin Test
1. Add 1 drop of red blood cells suspended to 2-4% in isotonic saline.
2. 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.

3. Add 2 drops of Anti-Human Globulin Anti-IgG to each test tube.
4. Mix the contents of the test tube well and centrifuge immediately. 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.
5. 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.
6. Record results.
7. To all negative tests add IgG sensitized red blood cells and follow manufacturer's instructions. 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 on each day of use and in accordance with local, state and federal regulations.

All negative antiglobulin tests should be controlled using IgG sensitized 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 tube tests 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

Anti-IgG		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	2187	0	2187	
	Negative	1	6610	6611	
	Total	2188	6610	8798	
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.

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

Further comparator tests were performed using Anti-Human Globulin Anti-IgG (Rabbit) Z357U (Green) as the test reagent and Anti-Human Globulin Anti-IgG (Rabbit) Z356U as the comparator reagent.

In total, 100 indirect antiglobulin tests were performed as follows:

Anti-IgG		Comparator Reagent		
		Positive	Negative	Total
Trial Reagent	Positive	20	0	20
	Negative	0	80	80
	Total	20	80	100
Positive Percent Agreement*				100
Negative Percent Agreement*				100

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

- Antibody Identification Process

Anti-IgG		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	416	0	416	
	Negative	0	350	350	
	Total	416	350	766	
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, 766 samples were tested for antibody identification. 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.

- ABO Cross-Match

Anti-IgG		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	119	0	119	
	Negative	0	221	221	
	Total	119	221	340	
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

Anti-IgG		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	303	0	303	
	Negative	0	545	545	
	Total	303	545	848	
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, 848 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

Anti-IgG		Comparator Reagent			One-sided 95% Exact lower confidence limit
		Positive	Negative	Total	
Trial Reagent	Positive	265	1	266	
	Negative	1	1253	1254	
	Total	266	1254	1520	
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 also 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.

Further comparator tests were performed using Anti-Human Globulin Anti-IgG (Rabbit) Z357U (Green) as the test reagent and Anti-Human Globulin Anti-IgG (Rabbit) Z356U as the comparator reagent.

In total, 25 direct antiglobulin tests were performed as follows:

Anti-IgG		Comparator Reagent		
		Positive	Negative	Total
Trial Reagent	Positive	5	0	5
	Negative	0	20	20
	Total	5	20	25
Positive Percent Agreement*				100
Negative Percent Agreement*				100

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

Precision Study Results

As part of the performance evaluation for both clear (Z356U) and green (Z357U) Anti-IgG (Rabbit), 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 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

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