

# POLYSPECIFIC ANTI-HUMAN GLOBULIN REAGENT

## Rabbit Anti-IgG+Monoclonal Anti-C3d

### Presentation

This reagent has been prepared by blending polyclonal anti-IgG (produced by immunisation of rabbits with purified whole human IgG) with monoclonal Anti-C3d obtained by in vitro culture of an IgM immunoglobulin secreting mouse hybridoma. The final protein concentration has been adjusted to 0.8% to 1.2% w/v with bovine serum albumin. The reagent has been coloured green by a mixture of Patent Blue and Ariavit Tartrazine. The reagent has been sterilised by filtration and contains 0.08 – 0.12% w/v sodium azide. This reagent complies with the recommendations contained in the latest issue of Volume III of the Guidelines for the Blood Transfusion Services in the United Kingdom, HMSO.

### Quality Control

1. Control the overall sensitivity of the indirect antiglobulin technique by including a weak IgG antibody with each batch of tests.
2. Add cells coated with IgG antibody to all negative direct and indirect antiglobulin tests.
3. A suitable negative control should be used.

### Reagents Required

Polyspecific Anti-Human Globulin Reagent – Supplied, Weak Anti-Rh, IgG Coated Cells

Phosphate Buffered Saline-Phosphate (10mM) Buffered 0.15M NaCl pH 6.8-7.2 (at +20°C).

LISS-Low Ionic Strength Solution-0.03M NaCl, 0.003M Na<sub>2</sub>HPO<sub>4</sub> buffer pH 6.7 and 0.24M glycine.

EDTA-K<sub>2</sub>H<sub>2</sub> EDTA 4.45%, 0.3% NaOH solution to pH 7.0-7.4.0. 1ml amounts (dried) per 1ml of serum.

Fresh serum-As a source of complement. This should be separated from red cells immediately after venesection and stored no longer than 24 hours at +4°C or one month at -20°C. The serum should be free from a typical antibodies.

A suitable negative control, e.g. AB Serum.

**USE:** Blood grouping reagents issued by Plasmatec Laboratories Ltd for use by an antiglobulin technique should be used in accordance with the package insert which accompanies the blood grouping reagent.

## INDIRECT ANTIGLOBULIN TEST for Antibody screening and identification

### A. Tube technique – Normal Ionic Strength

1. Prepare a 3% suspension of red cells in Phosphate Buffered Saline.
2. Place 100µl of serum in a suitable tube.
3. To a second tube add 100µl of the negative control.
4. Add 50µl of cell suspension to each tube.
5. Mix well and incubate at +36°C to +38°C for 45 minutes.
6. Wash the cells a minimum of three times with Phosphate Buffered Saline.
7. Using the dropper provided add two drops of Polyspecific Anti-Human Globulin Reagent.
8. Mix well and centrifuge the tubes at 130rcf for 60 seconds or at a suitably validated rcf and time.
9. Gently re-suspend the cell button and observe macroscopically and where necessary microscopically for agglutination.

### B. Tube Technique- Low Ionic Strength

1. Prepare a 1.5% suspension of washed red cells in LISS by washing the cells twice in PBS and once in LISS. Re-suspend the cells to 1.5% in LISS.
2. Place 100µl of serum in a suitable tube.
3. To a second tube add 100µl of the negative control.
4. Add an equal volume of cell suspension to each tube.
5. Mix well and incubate at +36°C to +38°C for 15 minutes.
6. Wash the cells a minimum of three times with Phosphate Buffered Saline.
7. Using the dropper provided add two drops of Polyspecific Anti-Human Globulin Reagent.
8. Mix well and centrifuge the tubes at 130rcf for 60 seconds or at a suitably validated rcf and time.
9. Gently re-suspend the cell button and observe macroscopically and where necessary microscopically for agglutination.

### C. Tube Technique – EDTA Two Stage

#### Stage 1

1. Prepare a 3% suspension of washed red cells in Phosphate Buffered Saline.
2. Inactivate residual complement in the test serum by the addition of 4mg EDTA per 1ml serum.
3. Place 100µl of inactivated serum in a suitable tube.
4. To a second tube add 100µl AB Serum.
5. Add 50µl of cell suspension to each tube.
6. Mix well and incubate at +36°C to +38°C for 60 minutes.
7. Wash the cells three times with Phosphate Buffered saline.
8. Add one volume of fresh serum as a source of complement.
9. Mix well and incubate at +36°C to +38°C for 15 minutes.

## Stage 2

1. Wash the cells a minimum of three times with Phosphate Buffered Saline.
2. Using the dropper provided add two drops of Polyspecific Anti-Human Globulin reagent.
3. Mix well and centrifuge the tubes at 130rcf for 60 seconds or at a suitable alternative rcf and time.
4. Gently resuspend the red cell button and observe macroscopically and where necessary microscopically for agglutination.

### D. Microplate Test–Normal Ionic Strength

1. Prepare a 3 % suspension of washed red cells in Phosphate Buffered Saline.
2. Place 70µl of serum into the well of a V well microplate.
3. Add 35µl 3% cell suspension (suitable positive and negative controls must be set up).
4. Agitate the plate to mix the reactants and incubate for 45 minutes at +36°C to +38°C.
5. Centrifuge the plate at 100rcf for 1 minute or at a suitably validated rcf and time.
6. Remove the supernatant and agitate the plate.
7. Wash the plate five times in Phosphate Buffered Saline.
8. Using the dropper provided add two drops of Polyspecific Anti-human Globulin Reagent to each well.
9. Agitate the plate and centrifuge at 100rcf for 60 seconds or at a suitably validated rcf and time.
10. Tilt the plate at an angle of 45 degrees and observe until the negative control has streamed and the positive control stays as a button in the V well.

### E. Microplate Test–Low Ionic Strength

1. Prepare a 3% suspension of washed red cells in LISS.
2. Place 35µl serum into the well of a V well microplate.
3. Add 35µl 3% cell suspension in LISS (suitable positive and negative controls must be set up).
4. Agitate the plate to mix the reactants and incubate for 15 minutes at +36°C to +38°C.
5. Centrifuge the plate at 100rcf for 1 minute or at a suitably validated rcf and time.
6. Remove the supernatant and agitate the plate.
7. Wash the plate five times in Phosphate Buffered Saline.
8. Using the dropper provided add two drops of Polyspecific Anti-human Globulin Reagent to each well.
9. Agitate the plate and centrifuge at 100rcf for 60 seconds or at a suitably validated rcf and time.
10. Tilt the plate at an angle of 45 degrees and observe until the negative control has streamed and the positive control stays as a button in the V well.

## DIRECT ANTIGLOBULIN TEST

Blood samples obtained for the direct antiglobulin test should be drawn into containers with EDTA as anticoagulant.

1. Prepare a 3% suspension of red cells in Phosphate Buffered Saline.
2. Place 50µl of cell suspension in a suitable tube.
3. Wash the cells three times with Phosphate Buffered Saline.
4. Using the dropper provided add two drops of Polyspecific Anti-Human Globulin reagent.
5. Mix well and centrifuge the tube at 130rcf for 60 seconds or at a suitable alternative rcf and time.
6. Gently resuspend the red cell button and observe macroscopically and where necessary microscopically for agglutination.
7. Incubate all weak or negative tests for 5 minutes at room temperature (limits 18°C to 22°C). Re-centrifuge tests and re-examine for agglutination.

## NOTES

- a. Inadequate washing of red cells in the anti-human globulin test may result in neutralisation of the anti-human globulin reagent.
- b. Care must be taken following the wash phase to remove residual saline: an excess of saline will dilute the reagent and reduce its efficacy.
- c. No single test is capable of detecting all clinically significant antibodies.
- d. Test serum for antibody screening or identification should be stored no longer than 24 hours at +4°C or 1 month at –20°C in order to detect complement binding antibodies. Serum stored at –20°C or below for longer periods will lose complement activity and may become anti complementary. Optimal results on stored serum may be obtained by a two stage sensitisation technique.
- e. Red cells specifically coated with C3d to control the anti-complement may be prepared in vitro.
- f. Centrifugation. Please note that the recommended centrifugation conditions are stated as rcf (relative Centrifugal Force). Please refer to your centrifuge manufacturer's instructions for equivalent rpm settings.
- g. Quality Control of this reagent has been performed using WASHED cells.
- h. For the tube techniques this reagent has been formulated and validated using glass tubes.
- i. All blood grouping reagents of human or animal origin are free from infectious agents. Appropriate care must be taken in the use and disposal of the container and its contents.
- j. DO NOT FREEZE: Store at +2°C to +8°C. Storage at temperatures outside this range may reduce the reactivity of the reagent.
- k. Use droppers provided and protect from contamination. The reagent must be used as supplied without dilution or addition.
- l. Do not use if the reagent exhibits turbidity or if a precipitate, gel or particles are present.

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