ANTI-HUMAN GLOBULIN

CONTENTS			
REF	3410010	Anti-Human Globulin	10 mL
For *in vitro* diagnostic use only			

AHG

Anti-human globulin polyspecific

Qualitative procedure for the detection of antibodies or components of human complement.

TUBE TESTS

PRINCIPLE

The antiglobulin test or Coombs Test, detects and identifies immunoglobulins bound to antigen sites of the red cell.
The addition of anti-human globulin serum will cause their agglutination.

APPLICATIONS

Indirect Antiglobulin Test
- Screening for unexpected antibodies
- Compatibility Testing
- Red cell phenotype
- Identification and titration of antibodies

Direct Antiglobulin Test
- Diagnosis of haemolytic disease of the newborn
- Diagnosis of haemolytic anaemia
- Investigation of suspected transfusion reactions

REAGENTS COMPOSITION

Anti-Human Globulin has been prepared by blending anti human anti-IgG and C3d monoclonal.
The stained reagent confirms at a glance that it has been added to all tubes.

ANTI-HUMAN GLOBULIN	Rabbit anti-human IgG. Murine Monoclonal anti-human C_3d. Stabilized buffer. Sodium azide <0.1% and dyes: Patent Blue and Tartrazine.

Precautions: The reagent and samples should be handled cautiously. Protective clothing should be worn, such as disposable gloves.

Warning: The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

REAGENT PREPARATION

The reagents are ready to use.

STORAGE AND STABILITY

1. The reagents will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use the reagents after the expiration date.
2. Do not freeze or expose to elevated temperatures. Prolonged storage outside the recommended temperature range may result in accelerated loss of reagent reactivity.
3. This product should be clear. Turbidity may indicate microbial contamination. Do not use the reagents if a precipitate is present.
4. If a vial is cracked or leaking, discard the contents immediately.

SAMPLES

Samples should be collected with or without an anticoagulant. The specimen should be tested as soon as possible after collection. Store at 2º-8ºC.
Serums less than 24 hours old kept at 2-8ºC since separation from the clot.
Blood specimens exhibiting gross haemolysis or contamination should not be used.

MATERIAL REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Pasteur pipettes.
- Water bath at 37ºC.
- Centrifuge Sero-fuge or similar.

ADDITIONAL REAGENT REQUIRED

- Phosphate Buffered Saline (PBS):
  8.5 to 9.0 g/L NaCl (0.145-0.154 mol/L) pH 7.0±0.2 at 22 ±1ºC.
- Test red cells. Negative Control (Red cells phenotype Rh negative coated with anti-D) y Positive Control (Red cells phenotype Rh positive coated with weak anti-D).

PROCEDURE

I. Indirect Antiglobulin test

1. Into a small test tube place 1 drop of the test serum.
2. Add 1 drop of a 3% suspension of test red cells, which have been washed minimum 1 time and resuspended in PBS.
3. Mix and incubate at 37ºC for 30-60 min.
4. After incubation, wash the cells three times with PBS. **Wash** (Note 1): fill the tube with PBS (approx. 4mL) and centrifuge the red cells allow adequate spin time sediment red cells, decant completely the supernatant

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es

Repeat the wash twice. Decant completely after the last washing.
5. Add 2 drop of Anti-human Globulin Reagent.
6. Mix well and centrifuge at 1000 r.c.f. or for a suitable alternative time and force (Note 2).

II. Direct Antiglobulin test
1. Prepare a 3% suspension of test red cells in PBS.
2. Into a small test tube place 1 drop of the red cell suspension.
3. Continue through stage 3-5 as specified in the I**ndirect anti globulin test.**

Reading (Note 3)
Dislodge the cell pack in the bottom of the tubes by gently tilting or shaking the tube and examine macroscopically for agglutination.
- *Negative reaction:*
  A smooth homogeneous resuspension of cells indicates a negative reaction.
- *Positive reaction:*
  Agglutination of the red cells indicates they are coated with an antibody.

Interpretation
- *Positive reaction*:
  Agglutination of the test red cells constitutes a positive test result, within accepted limitations of test procedure.
- *Negative reaction*:
  No agglutination of the test red cells constitutes a negative result, within the accepted limitations of the test procedure.

QUALITY CONTROLS

To confirm the validity of a negative result one-drop (50 µL) of washed cells coated with a weak IgG antibody (Coombs Control Cells) should be added to the tube, recentrifuged and examined for agglutination (See: See step 6 and Reading of Tube Test.) If no agglutination is observed the test is invalid and must be repeated.

ADVICE

Note 1. Complete washing steps without interruption.
Note 2. Centrifuge and read tests immediately after addition of anti-human globulin because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
Note 3. Read all tube tests straight after centrifugation.

ANALYTICAL PERFORMANCE

- The reagent has been characterized by all the procedures mentioned in the PROCEDURE.
- Prior to release, each lot of AGH is tested by the PROCEDURE against coated blood cells.

LIMITATIONS OF THE PROCEDURE

- Inadequate washing of the red cells, contamination with human serum protein of the anti-serum, incorrect incubation or centrifugation times and inadequate temperature ranges give false negative reactions.
- Test cells, which have a positive Direct Antiglobulin Test, will also give a false positive reaction with all Indirect Antiglobulin Tests.
- Test serum should be stored no longer than 24 hours at 2-8ºC or 1 month at –20ºC in order to detect complement-binding antibodies.
- The reagent will not agglutinate red cells coated with C4d fragments.
- No single test is capable of detecting all clinically significant antibodies.
- The reagent must be used as supplier without dilution or addition.
- This reagent has been formulated and validated using glass tubes.
- Use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use.

REFERENCES

- Coombs R.R.A., Mourant, A.E. and Race, R.R. Lancet ii:15 (1945).
- Coombs R.R.A., Mourant, A.E. and Race, R.R. Brit J. Exp. Path. 26:225 (1945).
- Pirofsky B. American Association of Blood Banks:59 (1972).

G3410-2/0407
R1.ing

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es