ERYCLONE®

ANTI-D (Rho)
(IgG)
MONOCLONAL BLOOD TYPING ANTIBODIES FOR SLIDE AND MODIFIED TUBE TESTS

SUMMARY
Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or are derived from a human cell line through EBV transformation. Each hybridoma cell line produces homogeneous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity.
Human red blood cells are classified as Rho(D) positive or Rho(D) negative depending upon the presence or absence of D(Rho) antigen on them. Approximately 85% of the Caucasian population is Rho(D) positive. The D^{u} phenotype is a variant of D(Rho) antigen and is recognised by performing the Antiglobulin test.

REAGENT
ERYCLONE®Anti-D (Rho) (IgG) is a ready to use high protein reagent prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation. These antibodies of the immunoglobulin class IgG are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognizing different epitopes of the human red blood cell antigen D (Rho).
Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY
a. Store the reagent at 2-8°C. DO NOT FREEZE.
b. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE
Human red blood cells possessing the D(Rho) antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with ERYCLONE® Anti-D(Rho)(IgG) reagent is a positive test result and indicates the presence of D(Rho) antigen. No agglutination with Anti-D(Rho)(IgG) reagent is a negative test result and indicates the absence of D(Rho) antigen. All negative test results should be further tested for D^{u} (Presence of weak / partial D's) by performing the D^{u} test procedure as described later.

NOTE
1. In vitro Diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. ERYCLONE®Anti-D (Rho) (IgG) reagent is not from human source, hence contamination due to HBsAg and HIV is practically excluded.
3. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.

SAMPLE COLLECTION AND PREPARATION
No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed samples.
Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period:
EDTA or Heparin - 2 days, Sodium citrate or sodium oxalate - 14 days, ACD or CPD - 28 days.

ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS
Glass slides (60 x 85 mm), Test tubes (12 x 75 mm), Pasteur pipettes, Isotonic saline, Centrifuge, Timer, Mixing sticks, ERYCLONE®Anti Human Globulin (Coombs) reagent, ERYCLONE®Rh-hr control.

TEST PROCEDURE
Slide Test
It is recommended that a negative control be run simultaneously with each RhoD test sample using ERYCLONE®Rh-hr control because invalid positive results may be obtained as with all high protein blood typing reagents, especially with samples having autoantibodies or abnormal serum proteins.
Bring reagent and samples to room temperature before testing.
Slide Test
1. Place one drop of ERYCLONE®Anti-D (Rho) (IgG) reagent on a clean prewarmed glass slide (40-45°C surface temperature).
2. Add one equal drop of whole blood.

3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm^2.
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at two minutes.

Tube Test

1. Prepare a 5% cell suspension of the red cells to be tested in isotonic saline.
2. Place one drop of ERYCLONE®Anti D (Rho) (IgG) reagent into a labeled test tube.
3. Pipette into the test tube, one drop of the 5% cell suspension and mix well. Incubate at 37°C for 15 minutes.
4. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button observing for agglutination macroscopically.

D^u TEST PROCEDURE

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of ERYCLONE®Anti D (Rho) (IgG) reagent into a labeled test tube.
3. Pipette into the test tube one drop of the 5% cell suspension under test and mix well. Incubate at 37°C for 15 minutes.
4. Wash the contents of the tube thoroughly, atleast three times, with isotonic saline and decant completely after the last wash.
5. Add two drops of ERYCLONE®Anti Human Globulin reagent and mix well.
6. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
7. Very gently, resuspend the cell button observing for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and Tube Tests

(a) Agglutination with reagent and no agglutination with Rh-hr control is a positive test result and indicates the presence of the D(Rho) antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination with reagent and control is a negative test result and indicates the absence of D(Rho) antigen. (b) Agglutination in Rh-hr (negative) control indicates the presence of autoantibodies or rouleaux formation. In such cases it is recommended that the determination of Rh factor should be made with saline reacting Anti-D such as RHOFINAL® Anti-D (IgM + IgG). (c) Cord cells heavily sensitized with Anti-D (Rho) may give false negative immediate spin test result.

D^u Test Procedure

(a) Agglutination with reagent and no agglutination with control indicates the presence of D^u antigen (weak / partial D's). No agglutination with reagent and control indicates the absence of D^u antigen. (b) Mixed field agglutination in the D^u test on red cells from a recently delivered woman may indicate a mixture of maternal Rho (D) negative and fetal Rho (D) positive blood. (c) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D^u antigen (weak / partial D's).

Note

1. It is strongly recommended that red cells with known Rh characteristics should be periodically run, preferably on a daily basis to validate the reagent performance.
2. The quality control of Anti-D IgG is performed using BSA replacement technique at 37ºC.
3. The reagents are expected to exceed minimum specifications /acceptance criteria as per label claim for titre, specificity & avidity as laid down by transfusion medicine technical manual -2003 with reference to NIBSC -UK & WHO international; standards.

REMARKS

As undercentrifugation and/or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required of achieving the desired results.

It is strongly recommended that as a routine quality control measure known as Rho (D) positive and Rho (D) negative red cells be occasionally run, preferably on a daily basis so as to control reagent performance and validation of test results. After usage the reagents should be immediately recapped and replaced to 2-8°C storage.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Lee H.H., Rouger P., Germain C., Muller A & Salmon C. (1983). The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on monoclonal antibodies. (2) Human Blood Groups by Geoff Daniels, 1st Ed., Blackwell Science, Oxford 1995. (3) HMSO, Guidelines for Blood Transfusion Services, 2nd Ed., 1994. (4) Quality Control of ABO and Rh blood grouping reagents " from NIB- INDIA. (5) Data on file: Tulip Diagnostics (P) Ltd.

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Manufactured by:

TULIP DIAGNOSTICS (P) LTD.

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In vitro Diagnostic Reagent
NOT FOR MEDICINAL USE.
Store at 2-8°C. DO NOT FREEZE.
Preservative: 0.1% NaN_3
An ISO 13485 Certified Company