Rubella IgG ELISA Kit
Qualitative/semi-quantitative assay for anti-Rubella IgG antibodies
Product code GD82
96 tests
For in vitro research use only

1. Intended use
The Rubella IgG kit is a rapid ELISA designed for the qualitative/semi-quantitative detection of IgG antibodies to Rubella virus in human serum. The assay is intended to be used to evaluate single sera for immune status or paired sera to demonstrate seroconversion, and is for research use only. Plasma samples may also be used.

2. Introduction
Rubella (German Measles) is a common and usually benign contagious disease of children and young adults. The primary medical significance comes from its teratogenic effects when contracted by childbearing women. Maternal infection, especially during the first trimester of pregnancy, can result in a range of congenital birth defects including deafness, cataracts, diabetes and cardiac and bone abnormalities. Because of the serious complications of the disease, it is important to determine the immune status of women of child bearing age, pregnant women, and individuals who may have close contact with them.

The presence of circulating maternal antibody indicates immunity to Rubella and virtually excludes the possibility of transmission of Rubella to the fetus. Acute Rubella infection can be confirmed by simultaneously testing paired acute and convalescent sera and looking for seroconversion, or by detecting Rubella specific IgM (GD83). The presence of Rubella specific IgM in the neonate or the persistence of a high titre of IgG antibody for longer than 6 months confirms a diagnosis of congenital Rubella.

A woman tested to be non-immune can be educated on the availability of vaccination. Pregnant women with current Rubella infection should be counselled on the consequences of congenital infection.

3. Principle of the test
Diluted serum or plasma specimens (1:100) are incubated for 20 minutes to allow specific antibodies to Rubella to bind to the antigen-coated wells. After washing away unbound antibody and other serum constituents, Rubella specific IgG is detected using rabbit anti-human IgG conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB enzyme substrate is added for 10 minutes. A blue colour develops if antibodies to Rubella are present. Addition of stop solution gives a yellow colour and the optical densities of controls and standards(s) and samples are measured using a microplate reader.

4. Materials included in the Kit
- Microplate 96 wells in 12 X 8 break-apart strips, pre-coated with inactivated Rubella virus antigen.
- Reagent 1: Sample Diluent 150mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 10ml, (blue), concentrate (x15)
- Reagent 2: Wash Buffer 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, concentrate (x10)
- Reagent 3: Conjugate (peroxidase conjugated rabbit anti-human IgG), 12 ml, (red), Ready to use
- Reagent 4: TMB Substrate, 12 ml, Ready to use
- Reagent 5: Stop solution, 12 ml. Ready to use
- Standards 1: 15 IU/ml (yellow), 25 IU/ml & 100 IU/ml (blue). Ready to use.
- Positive control 1: (50 IU/ml) 1ml liquid, (red). Ready to use.
- Negative control 1: (< 15 IU/ml) 1ml liquid, (green). Ready to use.
- Instructions for use

5. Other equipment required
- 10mm X 60mm tubes for dilution, pipettes 10µl, 100µl, 1000µl;
- repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device. Distilled or de-ionised water, general laboratory apparatus.

6. Storage and precautions
On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The assay standards and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stop solution contains 0.2M sulphuric acid and is non-corrosive.

7. Samples
Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid hemolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at – 20°C. Avoid multiple freeze-thaw cycles.

8. Method
Ensure that all materials are at room temperature before beginning the procedure. We recommend that the standards and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1. Assemble the number of strips required for the assay.
2. The sample diluent X15 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 – 8°C. Dilute the Sample Diluent (Reagent 1) 1:14 in distilled water to make sufficient buffer for the assay run e.g. add 10ml sample diluent concentrate to 140 ml water.
3. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml diluent). It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.
4. For qualitative determinations, dispense 100 µl of the negative control, the 15 IU/ml standard, the positive control and the diluted patient sample into the wells. For semi-quantitative determinations, use sample diluent as 0 IU/ml and additionally dispense the 25 IU/ml and 100 IU/ml standards.
5. Incubate for 20 minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
6. Dilute the Wash Buffer (Reagent 2) 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water. The diluted wash buffer is stable for two months at 2 - 8°C.
7. After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing is the key to good results. Blot the wells on absorbent paper before proceeding. Do not allow the wells to dry out.

Manual Wash Procedure:
Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process two more times.

8. Dispense 100µl of Conjugate (Reagent 3) into each well. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate. Incubate the wells for 20 minutes at room temperature.

9. After 20 minutes, discard the well contents and carefully wash the wells four times with wash buffer. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. Do not allow the wells to dry out.

10. Using a repeating dispenser, rapidly dispense 100µl of the TMB Substrate (Reagent 4) into each well. Incubate the plate for 10 minutes.

11. Add 100µl of Stop Solution (Reagent 5) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

12. Read the optical density (OD) in a microplate reader within 10 minutes.

9. Quality control
The expected optical density values for the negative and positive controls and the standards are given on the certificate included in the kit.

10. Interpretation
Qualitative determinations
Negative samples: OD < OD of 15 IU/ml standard
Positive samples: OD >/= OD of 15 IU/ml standard

Semi-quantitative determinations
Plot the optical densities of the standards against their respective concentrations. Draw a line to join the points. Read the concentrations of the unknowns from this graph. Concentrations above 15 IU/ml are considered positive for anti-Rubella IgG.

1. A negative result indicates susceptibility to primary infection. However, see Limitations below.
2. A positive result indicates a current or previous infection with Rubella virus. Individuals with current infection are considered to be at risk of transmitting Rubella virus infection. Positive individuals are considered immune to further infection.
3. To evaluate acute and convalescent sera, both samples must be tested in the same assay. If the acute specimen is negative and the convalescent specimen is positive, seroconversion has taken place and a primary Rubella virus infection is indicated.

11. Limitations
1. The antibody titre of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
2. Test results for demonstration of seroconversion should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
3. Samples collected too early in the course of the infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion or an IgM specific assay should be performed.
4. A positive Rubella IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection.

12. Expected Values
Seroprevalence studies indicate that in most countries, 80-90% of the adult population have detectable antibodies to Rubella.

13. Performance characteristics
Comparative study:
The Genesis Diagnostics Rubella IgG kit was compared with another commercially available ELISA procedure for the detection of IgG antibodies to Rubella virus. The Genesis kit showed 100% agreement with the other ELISA. The results are summarised below.

<table>
<thead>
<tr>
<th>Comparative Study (n=78)</th>
<th>Reference Rubella IgG ELISA kit</th>
</tr>
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<tbody>
<tr>
<td>Genesis Diagnostics</td>
<td>+</td>
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<tr>
<td>Rubella IgG kit</td>
<td>-</td>
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<tr>
<td></td>
<td>52 0</td>
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14. Assay characteristics
Within Assay Imprecision <12%
Between Assay Imprecision <12%

Further Reading