Version 20-12/24

**User's Manual**

# Measles IgG ELISA Kit

REF **DEIA359S**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

The Measles Virus IgG tests are quantitative and qualitative immunoassays for the detection of human antibodies in serum or plasma directed against Measles Virus. The Measles Virus IgG assay is recommended for determination of immune status and for detection of intrathecal synthesized IgG antibodies in cerebrospinal fluid.

## General Description

Measles Virus, in common with Mumps virus, Parainfluenza Viruses and RS Virus belongs to the family of paramyxoviridae and has a worldwide distribution. According to WHO estimates, one million individuals die as a consequence of Measles Virus infections every year. A number of countries, along with the WHO, had set themselves the goal of eradicating measles by a program of vaccination.

The highly contagious nature of the virus has ensured that the measles disease syndrome has long been established as a typical childhood infection. Before the introduction of vaccination the majority of children (95 – 98 %) had been infected by the age of ten. The epidemiology has changed since the widespread use of an effective vaccine was implemented and now cases of measles are more commonly seen in the late school and early adult age range.

## Principles of Testing

The ELISA is an immunoassay, which is particularly suited to the determination of antibodies in the field of infectious serology. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in the test serum sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colourless substrate p-nitrophenylphosphate is then converted into the coloured product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

## Reagents And Materials Provided

1. **ELISA Microplate:** 12 × 8

Break apart microtiter test strips each with eight antigen coated single wells.

The coating material is inactivated.

2. **Standard Serum (ready-to-use)** 2 × 2 ml

Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBsAg (Hepatitis B-Virus surface antigen) and anti-HCV Ab;

preservative: < 0.1 % sodium azide; colouring: Amaranth O.

3. **Negative Control Serum (ready-to-use)** 2 ml

Human serum in protein containing phosphate buffer; negative for anti-HIV Ab, HBsAg (Hepatitis B-Virus

surface antigen) and anti-HCV Ab;

preservative: < 0.1 % sodium azide; colouring: Lissamin Green V.

4. **Anti-Human IgG Conjugate (ready-to-use)** 13 ml

Anti-human IgG polyclonal antibody, conjugated to alkaline phosphatase, stabilised with protein stabilisation solution;

preservative: < 0.1 % methylisothiazolone, < 0.1 % bromnitrodioxane.

5. **Washing Solution Concentrate (sufficient for 1000 ml)** 33.3 ml

Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7.4;

preservative: < 0.1 % sodium azide.

6. **Dilution Buffer (ready-to-use)** 2 × 50 ml

Protein containing phosphate buffer with Tween 20;

preservative: < 0.1 % sodium azide; colouring: 0.01 g/l Bromphenol blue.

7. **Stopping Solution (ready-to-use)** 15 ml

< 0.1 N sodium hydroxide, 40 mM EDTA.

8. **Substrate (ready-to-use)** 13 ml

Para-nitrophenylphosphate in solvent free buffer;

preservative: < 0.1 % sodium azide.

## Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
3. Microtiter plate washer
4. Incubator 37°C
5. Moist chamber
6. Distilled water

## Storage

The kit is stored at 2 - 8°C (Avoid Direct Light), and not be frozen or thawed. The product is valid for 12 months. After opening, store at 2 - 8°C, it can be stable for 6 months, avoid contamination.

## Specimen Collection And Preparation

1. Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) or CSF collected according to standard laboratory methods are suitable samples. Serum and CSF samples from a test should be taken on the same day and should be analysed in parallel. Samples must not be thermally inactivated.

2. **Dilution of Samples**

Before running the test, samples ($V_1$) must be diluted in dilution buffer ($V_2$) as follows:

| | | | |
|---|---|---|---|
| $V_1 + V_2 = 1 + 100$ | add | 10 µl | test's sample |
| | each to | 1000 µl | dilution buffer |

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

3. **Sample Storage**

The samples should not be stored for more than 7 days at 2 – 8 °C. Extended storage is possible at -20 °C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2 – 8 °C for one week.

## Plate Preparation

The microtiter test strips labeled with abreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper resealing. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

## Assay Procedure

### Preparation of Kit Reagents

1. Bring all reagents to room temperature before testing.
2. Negative control and standard sera are ready-to-use and must not be diluted any further. For each test run - independent of the number of microtiter test strips to be used – negative control and standard sera must be included. Standard sera should be set up in duplicate. Do not treat negative control and standard sera with Rf-absorbent.
3. The required conjugate concentration (+, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label. Avoid contamination.
4. **Washing Solution (Concentrate)**

Dilute washing buffer concentrate ($V_1$) 1:30 with aqua dest. to a final volume of $V_2$. Bottles used for the working dilution should be cleaned regularly. Discard cloudy solutions. Example:

| Buffer concentrate ($V_1$) | Final volume ($V_2$) |
|---|---|
| 33.3 ml | 1000 ml |
| 1.0 ml | 30 ml |

5. **Dilution Buffer for Samples (ready-to-use)**

Discard cloudy solutions.

6. **Substrate (ready-to-use)**

Substrate in unopened bottle may have a slightly yellow coloring, which does not reduce the quality of the

product! Avoid contamination.

7. **Stopping Solution (ready-to-use)**

**Manual Test Procedure**

1. Place the required number of cavities in the frame and prepare a protocol sheet.
2. Add each 100 µl of diluted sample or ready-to-use negative control/standard sera into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

| Well | Quantitative ELISA |
|---|---|
| A1 | substrate blank |
| B1 | negative control |
| C1 | standard serum |
| D1 | standard serum |
| E1 | patient 1 ... |
| F1 | patient 2 ... |

3. Sample incubation for 60 minutes (+/- 5 min.) at 37°C (+/- 1°C) in moist chamber
4. After incubation wash all wells with washing solution (by automated washer or manually): - aspirate or shake out the incubation solution - fill each well with 300 µl washing solution - aspirate or shake out the washing buffer - repeat the washing procedure 3 times (altogether 4 times!) - dry by tapping the microtiter plate on a paper towel
5. Addition of conjugate Add 100 µl of the ready-to-use IgG conjugate to the appropriate wells (except substrate blank)
6. Conjugate incubation for 30 minutes (+/- 1 min.) at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above).
8. Addition of substrate Add 100 µl of ready-to-use substrate solution to each well (including well for substrate blank!)
9. Substrate incubation for 30 minutes (+/- 1 min.) at 37°C (+/- 1°C) in moist chamber. Ensure dark incubation.
10. Stopping of the reaction Add 100 µl stopping solution to each well, shake microtiter plate gently to mix.
11. Read extinction Read optical density (OD) within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

## Interpretation Of Results

For the ELISA test evaluation a lot-specific quality control certificate with standard curve and an evaluation table is included in the test kit so that the obtained OD values may be assigned to the corresponding antibody activities. The substrate blank must be subtracted from all OD values prior to evaluation. Mean OD value of the standard serum (STD), tested in duplicate, has to be used.

**Method 1:**

In the first line of the table, several ranges of OD values for the standard serum are depicted covering the

whole standard validity range. According to the measured mean OD value of the standard serum, the corresponding column can be chosen. This column contains the information of upper and lower cut-off OD values to allow evaluation of the sample. OD values below the lower cut-off are evaluated negative and values above the upper cut-off are evaluated positive. Implementation of the correction factor F is not necessary in the context of the evaluation table.

**Method 2:**

Qualitative Evaluation

To fix the cut-off ranges multiply the mean value of the measured standard OD with the numerical data of the quality control certificate (see special case formulas), e.g.:

OD = 0.502 x MW(STD) with upper cut-off

OD = 0.352 x MW(STD) with lower cut-off

If the measured mean absorbance value of the standard serum is 0.64 OD, the range of the cut-off is in between 0.225-0.321 OD.

Calculation example:

Standard serum mean OD = 0.64

Upper cut-off: OD = 0.502 x 0.64 = 0.321

Lower cut-off: OD = 0.352 x 0.64 = 0.225

**Criteria of Validity**

1. The substrate blank must be < 0.25 OD.
2. The negative control must be negative.
3. The mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific quality control certificate.
4. The variation of OD values of the standard serum must not be higher than 20 %.
5. If these criteria are not met, the test is not valid and must be repeated.
6. A positive test result confirms the presence of specific antibodies. A negative result indicates that no clinically relevant antibodies against the pathogen are present in the test´s sample, but does not exclude the possibility of an acute infection. In case of a borderline result a reliable evaluation is not possible. A definitive diagnosis can only be achieved by testing paired serum samples, taken at one to two weeks intervals, in parallel.
7. A positive IgM result is indicative of an acute measles infection. In the absence of manifest clinical symptoms of measles infection the detection of specific IgG indicates a past infection or a successful immunization.
8. Cross-reactions with antibodies directed against other viruses of the family paramyxoviridae have been reported.

## Evaluation

The mathematical curve fitting for antibody quantification with the immunoassays is based on the 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln(\frac{D-A}{OD(Sample)*F-A}-1)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A: Lower asymptote (OD)

Parameter B: Slope of the curve

Parameter C: Inflection point

Parameter D: Upper asymptote (OD)

For the adaption of the test level to the given 4 PL standard curve the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ STD\ OD\ value}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly evaluated from the 4 PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, the evaluation of antibody activities from the optical measurement signals (OD) of samples can be performed with the 4PL function presented above.

The determination of the IgG antibody activity in mIU/ml is based on the 2nd International Standard for Anti-Measles (NIBSC code: 66/202) and the 3rd International Standard for Anti-Measles (NIBSC code: 97/648) of the World Health Organization (WHO).

## Performance Characteristics

To evaluate the performance characteristics of the Measles Virus IgG, 195 serum samples from seronegative donors, patient samples, sera from children and unselected samples were examined in comparison to Measles virus IgG ELISA test system from two other manufacturer. Analyses have been performed according to the corresponding instructions for use. Borderline results were not included in the calculation of sensitivity and specificity.

Sensitivity: > 99 %

Specificity: > 95 %

Reproducibility:

| Sample | Mean Value (OD) | Intraassay CV (%) | Mean Value (OD) | Interassay CV (%) |
|---|---|---|---|---|
| Serum 1 | 0.166 | 2.9 | 1.218 | 3.6 |
| Serum 2 | 0.450 | 1.9 | 0.793 | 5.9 |
| Serum 3 | 1.144 | 1.7 | 0.257 | 5.5 |

## Detection Range

The borderline ranges of the Measles Virus IgG tests are specified on the quality control certificates and indicate the range of borderline test results. Values below this range indicate a negative test result; values above the borderline range are interpreted positive.

## Specificity

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with Measles Virus IgG and a commercially available anti-Measles Virus IgG ELISA. Positive sera (10 sera each) for Respiratory Syncytial Virus IgG, Mumps Virus IgG and Epstein-Barr Virus IgG have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation, reactivities were observed which are mainly due to the high vaccination rate and were confirmed by the reference test. Other cross-reactivities cannot be ruled out in general.

## Linearity

The limits of quantification are specified on the quality control certificate of the Measles Virus IgG. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. In case a patient sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

## Interferences

To determine the influence of interfering substances, sera with different reactivities were analyzed with Measles Virus IgG. No interferences have been detected for sera with concentrations up to 2.00 g/L hemoglobin, 11.50 g/L lipemia/triglyceride or 0.201 g/L bilirubin (conjugated and unconjugated).

## Precautions

1. The ELISA kit is designed for use by qualified personnel who are familiar with good laboratory practice.
2. All kit reagents and human specimens should be handled carefully, using established good laboratory practice.
3. This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV-ab, HBs-Ag (Hepatitis B-Virussurface Antigen) and anti-HCV-ab, they should be considered potentially infectious.

4. Do not pipette by mouth.
5. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
6. Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.
7. Patient's material and other potentially infectious material should be decontaminated after the test run.
8. Reagents should be stored safely and be inaccessible to unauthorized access e.g. children.

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