Version 09-08/25

User's Manual

Parvovirus B 19 IgG ELISA kit

REF **DEIA10283**

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 Parvovirus B19 IgG (Recombinant) Enzyme Immunoassay Kit measures IgG-class antibodies to Parvovirus B19 in human serum or plasma.

General Description

Parvovirus B19 causes Erythema infectiosum. A Parvovirus B19 infec- tion during pregnancy may result in spontaneous abortion, still birth or Hydrops fetalis in sero-negative pregnant women. Parvovirus B19 IgG antibodies are retained for life after contact with the virus. Parvovirus B19 IgM-antibodies can be detected at the earliest approx. 10 days after contact with the virus.

Principles of Testing

Highly purified Parovirus B19 antigens (a specific part of the VP1 produced by recombination as well as recombinant, eukaryote VP2 particles) are fixed in the wells of the microtitre plate.

1. Diluted serum or plasma samples are incubated in the wells, with antibodies binding specifically to the pathogen antigens coating the surface of the wells.
2. Unbound antibodies are then flushed away.
3. In a second step, anti-human immunoglobulin antibodies (IgG and / or IgM), which are coupled to horseradish peroxidase, are incubated in the wells.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected by a peroxidasecatalysed colour reaction. If an antigen/antibody-reaction occurs, the chromogen substrate solution colours proportionally to the quantity of the bound anti-Parvovirus B19 IgG antibodies. The intensity of the staining can be measured with a photometer and then conclusions can be drawn concerning the concentration of the anti-Parovirus B19 antibodies in the sample.

Reagents And Materials Provided

The reagents in one package are sufficient for 96 tests. Each test kit contains:

1. 12 x 8 wells **Microtitre plate** (cap strip marked in red) coated with recombinant Parvovirus B19 antigen in a self-sealing vacuum bag.
2. 450 µl **positive control** (violet cap) contains MIT (0.1%) and Oxypyrion (0.1%)
3. 450 µl **cutoff control** (yellow cap) contains MIT (0.1%) and Oxypyrion (0.1%)
4. 450 µl **negative control** (white cap) contains MIT (0.1%) and Oxypyrion (0.1%)
5. 500 µl **anti-human IgG conjugate** (101-times concen- trated, red cap) contains NaN3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)
6. 100 ml **Wash buffer** (ten times concentration) Contains phosphate buffer, NaCl, detergent, preserva- tive: MIT (0.01%) and Oxypyrion (0.1%)
7. 125 ml **Dilution Buffer** (ready-to-use) Contains protein, detergent and blue dye. Preservative: MIT (0.01%)

and Oxypyrion (0.1%)

8. 12 ml **Chromogenic substrate** tetramethylbenzidine (TMB, ready-to-use)
9. 12 ml **stop solution** 24.9% phosphoric acid (H_3PO_4) (ready for use)
10. 1 Instructions for use
11. 1 Evaluation form
12. 2 pieces of covering film

Materials Required But Not Supplied

1. Deionised water (high quality)
2. Test tube
3. Vortex mixer or other rotators
4. 8-channel pipette or washer with pump
5. Clean measuring cylinders, 50 ml and 1000 ml
6. Micropipettes with disposable tips, 10 µl and 1000 µl
7. 10 ml pipette or dispenser
8. Incubation chamber 37°C
9. Microtitre plate photometer
10. Timer
11. Disposable protective gloves
12. Waste container for bio-hazardous materials

Storage

Store reagents at +2°C to +8°C before and after use, do not freeze.

Specimen Collection And Preparation

The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid Microbial contamina- tion of the samples. Insoluble substances must be removed from the sample prior to incubation.

The use of heat-inactivated, icteric, haemolytic, lipemic or turbid samples is not recommended.

Caution!

If the tests are not conducted immediately, the sample can be stored for up to 2 weeks at +2°C to +8°C. Prolonged storage of the samples is possible at -20°C or below. Repeated freezing and thawing of samples is not recommended due to the risk of pro- ducing inaccurate results. Avoid more than 3 cycles of freezing and thawing.

Reagent Preparation

The test reagents are sufficient for 96 test runs. The following quantity specifications relate to the processing of a single microtitre plate strip with 8 wells respectively. While using several microtitre plate strips, the specified quantities must be simultaneously multiplied with the number of used microtitre plate strips respectively. The device-specific dead volume must be taken into account. The dilution buffers, substrate and stop solution are ready to use.

1. **Preparation of ready-to-use wash buffer**

The wash buffer concentrate is diluted 1 + 9 with H2O (deionised water). 5 ml concentrate is mixed with 45 ml H2O (deionised water) per microtitre plate strip with 8 wells. The ready-to-use wash buffer can be stored for four weeks at +2°C to +8°C or for one week at room temperature.

2. **Preparation of conjugate solution**

For each microtitre plate strip with 8 wells, 1 ml of dilution buffer and

10 µl of anti-human IgG peroxidase conjugate (red cap) or IgM peroxi- dase conjugate (green cap) are transferred to a clean container and

mixed well (dilution 1 + 100). The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate

solution.

Assay Procedure

1. Expose all reagents for at least 30 minutes to + 18°C to +25°C (room temperature) before beginning the test.
2. **Preparing samples and controls**

Pipette 10 µl sample and / or control to every 1 ml dilution buffer and mix well (dilution 1 + 100).

3. **Incubation of samples**

Pipette 100 µl of diluted sample and/or diluted control into each well and incubate for 1 hour at +37°C.

4. **Washing**

4.1 Carefully remove the covering film.

4.2 Completely empty the wells

4.3 Fill each of the wells with 300 µl of ready-to-use wash buffer

5. **Incubation with conjugate**

Add 100 µl of diluted conjugate solution and incubate for 30 minutes at +37°C.

6. **Washing**
7. **Substrate reaction**

Pipette 100 µl of ready-to-use

substrate solution into each well and incubate for 30 minutes at room temperature. The time is calculated from pipetting into the first well.

8. **Stopping the reaction**

Pipette 100 µl of ready-to-use stop solution into each well.

9. **Measurement of the extinction values**

The extinction values of the single wells are measured in a microtitre plate photometer at 450 nm and the reference wave length 650 nm (620 to 650 nm permitted).

Quality Control

1. The single extinction values of the double analysis of the cutoff control do not deviate by more than 20 % from their average.
2. Negative control extinction value ≤ 0.150
3. Cut-off control extinction value - Negative control extinction value ≥ 0.050 (E_{Cutoff} - $E_{neg.\ controls}$ ≥ 0.050)
4. Positive control extinction value - Cutoff control extinction value ≥ 0.300 ($E_{pos.\ contr.}$ - E_{Cutoff} ≥ 0.300)

These checks are used to validate the test results as per the "Valida- tion Quality Control" chapter. The reproducibility of results can be improved by determining the specific antibodies relative to the cut-off check in U/ml, as the fluctuations from the performance of the test are also included. In validating the test, the positive and negative checks do not need to be evaluated. If necessary, however, they can be carried out for internal quality control purposes. In this case, the results should lie within the target value range given in the analysis certificate or on the label.

Calculation

Cutoff (limit) = the average is formed from the extinction values of both cutoff controls (at the beginning and at the end of the series).

Qualitative evaluation

Grey range:

lower range = cutoff

upper range = cutoff + 20% (cutoff x 1.2)

Negative: Samples with extinction values below the grey range

Borderline: Samples with extinction values within the grey range

Positive: Samples with extinction values above the grey range

Quantitative evaluation

The corresponding antibody activity in units per ml is assigned to the extinction values using a formula. The measurement units U/ml are arbitrary units, which do not allow conclusions concerning (interna- tional) reference values.

U/ml sample (Extinction sample / extinction cutoff) x 20

Grey range:

lower range = 20 U/ml

upper range = 24 U/ml

Negative: U/ml sample < 20

Borderline: 20 ≤ U/ml sample ≤ 24

Positive: U/ml sample > 24

Samples with a borderline test result should be retested. If the results are still borderline after the second test, a further sample should be taken and tested after some time.

The linearity of the test was determined during the evaluation within the following range:

20 U/ml to 125 U/ml (R^2 = 0.95)

In case of an extinction ≥ 3.0 or a measuring value above the linear range, the result should either be given as > 125 U/ml, or the sample may be diluted and measured again. We recommend to start with a final sample dilution of 1:500 and if necessary further subsequent dilution steps.

Performance Characteristics

Sensitivity 100% (295/295)*

Specificity 98.7% (149/151)*

Samples from routine practice, samples from pregnant women and samples of children with acute Parovirus B-19 infection were tested to calculate the sensitivity and specificity. The data were compared with another, commercially available ELISA that also works with VP2 particles.

Interferences: Control studies on potential interfering factors have shown that the performance of the test is not affected by anticoagu- lants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia of the sample.

Cross-reactions: Potential interference from antibodies against EBV, as well as from other conditions that are due to atypical behaviour of

the immune system (anti-nuclear auto-antibodies, rheumatoid factor [positive after RF absorption) can be practically ruled out.

Sensitivity

Analytical sensitivity

WHO standard* ≈ 2 IU/ml Result: borderline (20 ≤ U/ml sample ≤ 24)

WHO standard* ≈ 3 IU/ml Result: positive (U/ml sample > 24)

WHO standard* 50 IU/ml Extinction: 2.5

WHO standard* 100 IU/ml Extinction: 2.8

* WHO standard (NIBSC code 93/724) was obtained from the National Institute for Biological Standards and Control (Hertfordshire, UK).

Precautions

1. Subject all ingredients to room temperature (+18°C to +25°C) for at least 30 minutes before beginning the test.
2. The control serums and conjugates are batch-dependent and may not be used across the whole range of parameters or batches.
3. Mix the concentrated conjugates, controls and patient samples thoroughly before use. Avoid the build-up of foam.

4. The covering films are intended for single use only.
5. The packages bear an expiry date. Once this has been reached, no guarantee of quality can be offered.
6. Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
7. The test should only be carried out by trained and authorised personnel.
8. Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add the patient samples and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells.
9. We have not validated these tests for screening of blood, blood components, cells, tissues, organs or any of their derivatives in order to assess the suitability for transfusion, transplantation or cell administration.
10. All blood products must be treated as potentially infectious.
11. The microtitre wells have been coated with inactivated whole cell lysates, bacterial or viral antigens.
12. After the addition of patient or control specimens, the microtitre wells must be considered to be potentially infectious and handled accordingly.
13. Donors' blood, in which no antibodies against HIV 1/2, HCV and hepatitis Bs antigen have been detected, is used for the manufacture of the control material. The control material must be treated with the same care as a patient sample, as infection cannot be excluded with total certainty.
14. Suitable disposable gloves must be worn throughout the entire test procedure.
15. The conjugates contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion, chloroazetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.
16. Phosphoric acid is an irritant. It is mandatory to avoid contact with skin and mucous membranes.
17. All fluids to be disposed of must be collected. All collecting containers must contain suitable disinfectants for the inactivation of human pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations.
18. The concentra- tions and incubation periods stated by the manufacturer must be observed.
19. Only use microtitre wells once.
20. Do not substitute or mix the reagents with reagents from other manufacturers.
21. Read through the entire instructions for use before carrying out the test and follow them carefully. Deviation from the test protocol pro- vided in the instructions for use can lead to erroneous results.