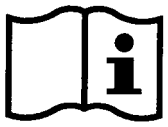
Product information

Information about other products is available at: www.ibl-america.com



Userś Manual

Parvovirus B19 IgM ELISA

Enzyme immunoassay for the determination of IgM-class antibodies to Parvovirus B19 in serum or plasma

REF **IB79807**

Σ **96 Wells**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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JL
Updated 221205

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Contents

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1 INTRODUCTION

The IBL-America Parvovirus B19 IgM Enzyme Immunoassay Kit provides materials for the determination of IgM-class antibodies to Parvovirus B19 in human serum or plasma (EDTA, Li-heparin, or citrate plasma).
For Research Use Only – Not for Use in Diagnostic Procedures

2 PRINCIPLE OF THE TEST

The IBL-America Parvovirus B19 IgM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA).

This ELISA is using a RF-Sorbent. The Rheumatoid factor (RF) is a special autoantibody form. These are autoantibodies, which are directed against the Fc fragment of human IgG. The RF autoantibodies are mostly class IgM, but may also be class IgA, IgG or IgE. The use of anti-human IgG antibodies in the RF-sorbent prevents false positive or false negative results.

Samples are diluted with Sample Diluent and additionally incubated with IgG-RF-Sorbent to remove rheumatoid factors. This pretreatment avoids false negative or false positive results.

Microtiter wells as a solid phase are coated with recombinant Parvovirus B19 antigen (VP1-s and VP2-s protein).
Pre-treated samples and **ready-for-use controls** are pipetted into these wells. During incubation Parvovirus B19-specific antibodies of positive samples and controls are bound to the immobilized antigens.
After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies are dispensed into the wells. During a second incubation this anti-IgM conjugate binds specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes.
After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.
The intensity of this color is directly proportional to the amount of Parvovirus B19-specific IgM antibody in the patient specimen. Optical density at 450 nm is read using an ELISA microtiter plate reader.

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3 PRECAUTIONS

- This kit is for research use only.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- Never pipette by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
- Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-America.

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4 KIT COMPONENTS

4.1 Contents of the Kit

1. **SORB MT Microtiter wells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with Parvovirus B19 antigen (VP1-s and VP2-s protein) (incl. 1 strip holder and 1 cover foil)
2. **SAM DIL Sample Diluent ***, 1 vial, 100 mL, ready to use, colored yellow, pH 7.2±0.2
3. **IgG-RF SORB IgG-RF-Sorbent*,** 1 vial, 6.5 mL, ready to use, colored yellow; Contains anti-human IgG-class antibody.
4. **CAL A Neg. Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.
5. **CAL C Pos. Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, red cap.
6. **CAL B Cut-off Control ***, 1 vial, 2.0 mL, ready to use; colored yellow, black cap.
7. **ENZ CONJ Enzyme Conjugate ***, 1 vial, 20 mL, ready to use, colored red, antibody to human IgM conjugated to horseradish peroxidase.
8. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
9. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.2 mol/l H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.
10. **WASH SOLN 20x Wash Solution ***, 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1 see „Preparation of Reagents“.

*contain non-mercury preservative

4.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450/620nm ±10 nm)
- Calibrated variable precision micropipettes
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Timer
- Absorbent paper

4.2 Storage and stability of the Kit

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for four months if stored as described above.

4.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution
Add fresh and germ-free distilled water to the 20X concentrated Wash Solution.
Dilute the complete content of the vial 1 + 19 (30 mL Wash Solution + 570 mL distilled water) to a final volume of 600 mL.
If crystals have formed in the wash solution concentrate, ensure that they are completely transferred and dissolved into the solution.
This diluted wash solution must have a pH value of 7.2 ± 0.2.
The diluted Wash Solution is stable for 1 week at 2 °C to 8 °C..

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

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4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-America has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SAMPLE

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay.
Please note: Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use hemolytic, icteric or lipaemic specimens. For further information, refer to chapter “Interfering Substances”.

5.1 Sample Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Subjects receiving anticoagulants may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Sample Storage and Preparation

Samples should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.
Samples held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution

Prior to assaying each sample is first to be diluted with *Sample Diluent*. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with *IgG-RF-Sorbent*

1. Dilute each sample **1+50** with *Sample Diluent*;
   e.g. 10 µL of sample + 0.5 mL of *Sample Diluent*. **Mix well.**
2. Mix well the IgG-RF-Sorbent before use.
3. Dilute this prediluted sample **1+1** with *IgG-RF-Sorben*t
   e.g. 60 µL prediluted sample + 60 µL *IgG-RF-Sorbent*. **Mix well**
4. **Let stand at room temperature for at least 15 minutes, up to a maximum of 2 hours and mix well again.**
5. Take 100 µL of these pretreated samples for the ELISA.

Please note: *Controls* are ready for use and must not be diluted!

6 ASSAY PROCEDURE

6.1 General Remarks

- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- During 37 °C incubation cover microtiter strips with foil to avoid evaporation.

6.2 Test Procedure

Prior to commencing the assay, dilute *Wash Solution*, **prepare samples as described in point 5.3** and establish carefully the **distribution and identification plan** supplied in the kit for all samples and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.
   Please allocate at least:
   1 well (e.g. A1) for the *Neg. Control,*
   2 wells (e.g. B1+C1) for the *Cut-off Control* and
   1 well (e.g. D1) for the *Pos. Control*.
   It is left to the user to determine controls and samples in duplicate.
2. Dispense
   100 µL of *Neg. Control* into well A1
   100 µL of *Cut-off Control* into wells B1 and C1
   100 µL of *Pos. Control* into well D1 and
   100 µL of each p r e a t r e a t e d sample with new disposable tips into appropriate wells.
3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C.**
4. Briskly shake out the contents of the wells. Rinse the wells **5 times** with diluted *Wash Solution* (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense **100 µL** *Enzyme Conjugate* into each well.
6. Incubate for **30 minutes at room temperature (20 - 25 °C).** *Do not expose to direct sun light!*
7. Briskly shake out the contents of the wells. Rinse the wells **5 times** diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add **100 µL** of *Substrate Solution* into all wells.
9. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.
10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well. Any blue color developed during the incubation turns into yellow. **Note:** Highly positive samples can cause dark precipitates of the chromogen!
11. Measure the optical density (OD) of the solution in each well at 450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended) with a microtiter plate reader.
    It is recommended that the wells be read within 30 minutes after adding the Stop Solution.

6.3 Measurement

Measure the optical density of all wells **at 450 nm** and record the OD values for each control and sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable **calculate the mean absorbance values** of all duplicates.

7 RESULTS

7.1 Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

***Neg. Control* in A1:**	Absorbance value **lower than 0.200**.
***Cut-off control (CO)* in B1/C1 :**	Absorbance value **between 0.350 – 0.850**
***Pos. Control* in D1**	Absorbance value **greater than 0.650 – 3.000**

The OD value of the Pos. Control should be higher than the OD value of the Cut-off Control. (OD Pos. Control > OD Cut-off Control).

7.2 Calculation

Mean absorbance value of Cut-off Control [CO]

Calculate the mean OD value of the duplicate determination of the Cut-off Control (e.g. in B1/C1).

Example: (0.44 + 0.45) ÷ 2 = 0.445 = CO

8 QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above-mentioned items without finding any error contact your distributor or IBL-America directly.

9 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.63 - 60 DU/mL.

9.2 Specificity of Antigen (Cross Reactivity)

No cross reactivity was found for Herpes-simplex Virus 1 and 2, Varicella zoster Virus and Epstein-Barr Virus (VCA), RSV, Rubella Virus, CMV and TBE.

9.3 Analytical Sensitivity

The analytical sensitivity of the IBL-America ELISA was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 0.63 DU/mL (OD_{450} = 0.034).

9.4 Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. (Detected by method comparison with Mikrogen ELISA, with three lots of IBL-America ELISA. 64 samples, therefrom 42 negative samples are assayed)

It is 100% for all three IBL-AMERICA production lots.

9.5 Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. (Detected by method comparison with Mikrogen ELISA, with three lots of IBL-America ELISA. 64 samples, therefrom 22 positive samples are assayed)

It is 100% for all three IBL-AMERICA production lots.

9.6 Method Comparison

The IBL-America Parvovirus B19 IgM ELISA was compared with the Mikrogen Parvovirus B19 IgM ELISA. 64 serum samples are assayed.

n=	64	Mikrogen	
		pos.	neg.
IBL-America ELISA	pos.	22	0
	neg.	0	42

Agreement: 100%reproducibility

9.6.1 Intra-assay

The intra-assay (within-run) precision of the IBL-America Parvovirus B19 IgM ELISA was determined by 20 x measurements of 12 serum samples covering the whole measuring range.

Sample	Mean OD_{450}	Intra-Assay CV (%)	n
1	0.20	4.72	20
2	0.23	4.14	20
3	0.09	6.24	20
4	1.06	4.21	20
5	0.91	3.45	20
6	0.90	4.22	20
7	1.45	3.84	20
8	1.48	2.22	20
9	1.27	4.29	20
10	2.38	3.24	20
11	3.13	2.14	20
12	1.82	2.75	20

9.6.2 Inter-assay

The inter-assay variation of the IBL-AMERICA Parvovirus B19 IgM ELISA was determined with 3 samples with 2 production kits in 10 independent runs with 2 replicates per run.

Sample	Mean OD_{450}	Inter-Assay CV (%)	n
1	3.03	6.29	40
2	1.97	14.68	40
3	1.57	8.40	40

10 LIMITATIONS OF USE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values. In immunocompromised subjects and newborns serological data only have restricted value.

10.1 Interfering Substances

Hemoglobin (up to 4.0 mg/mL), bilirubin (up to 0.5 mg/mL) and triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

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11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-America.

11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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SYMBOLS USED WITH IBL AMERICA ELISAS

Symbol	English	Deutsch	Français	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´ arresto
Zero Standard	Zero Standard	Nullstandard	Standard 0	Estándar 0	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Pos. Control	Positive Control	Positive Kontrolle	Positif Contrôle	Control positivo	Controllo positivo
Neg. Control	Negative Control	Negative Kontrolle	Négatif Contrôle	Control negativo	Controllo negativo
Cut-off Control	Cut-off Control	Grenzwert-Kontrolle	Valeur limite Contrôle	Control valor limite	Controllo valore limite
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
IgG-RF-Sorbent	Rheumatoid factor-Absorbent	Rheumafaktor-absorbens			Assorbente IgG-RF
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante

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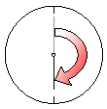
SHORT INSTRUCTIONS FOR USE

	All reagents and samples must be allowed to come to room temperature (18-25°C) before use.
	Dispense 100 µl of Controls into appropriate wells.
	Dispense 100 µl of sample into selected wells. **(Please note special sample treatment, point 5.3!)**
60 min 	Cover wells with foil. Incubate for **60 minutes** at 37 °C.
5x 	Briskly shake out the contents of the wells. **Rinse** the wells **5 times** with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets
	Dispense 100 µl of Enzyme-Conjugate into each well.
30 min 	Incubate for **30 minutes** at room temperature.
5x 	Briskly shake out the contents of the wells. **Rinse** the wells **5 times** with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets
	Add 100 µl of Substrate Solution to each well.
15 min 	Incubate for **15 minutes** at room temperature.
	Stop the reaction by adding 100 µl of Stop Solution to each well.
	Determine the absorbance of each well at 450 nm.

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