PARVO IgG-EIA "SEIKEN"

An enzyme immunoassay kit for qualitative determination of IgG antibody to human parvovirus B19

Enzym-Immunoassay-Kit zur qualitativen Bestimmung von IgG-Antikörpern gegen Humanes Parvovirus B19

Kit di dosaggio immunoenzimatico per la determinazione qualitativa di anticorpi IgG anti-parvovirus B19 umano

Coffret pour le dosage immuno-enzymatique de l'anticorps anti-IgG du Parvovirus B19 humain.

CE IVD

Symbols

Batch code
Chargenbezeichnung
Code du lot
Codice del lotto

Use by
Verwendbar bis
Utiliser jusque
Utilizzare entro

Contains sufficient for <n> tests
Ausreichend für "n" Ansätze
Contenu suffisant pour "n" tests
Contenuto sufficiente per "n" saggi

In Vitro Diagnostic Medical Device
In Vitro Diagnostikum
Dispositif médical de diagnostic in vitro
Dispositivo medico-diagnostico in vitro

Temperature limitation (Store at)
Zulässiger Temperaturbereich (Aufbewahrung bei)
Limites de température (Conservation à)
Limiti di temperatura (Conservare a)

Catalogue number
Bestellnummer
Référence du catalogue
Numero di catalogo

Contents of kit
Inhalt der Packung
Contenu du coffret
Contenuto della confezione

Consult Instructions for Use-
Gebrauchsanweisung beachten
Consulter les instructions d'utilisation
Consultare le istruzioni per l'uso

PARVO IgG-EIA "SEIKEN"

An enzyme immunoassay kit for qualitative determination of IgG antibody to human parvovirus B19

96 tests

INTENDED USE

To qualitatively detect the presence of human parvovirus B19 IgG antibodies in serum or plasma specimens.

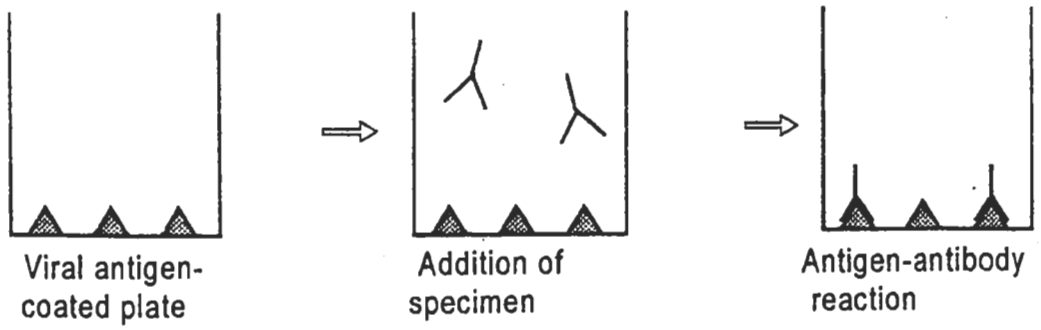
SUMMARY AND EXPLANATION

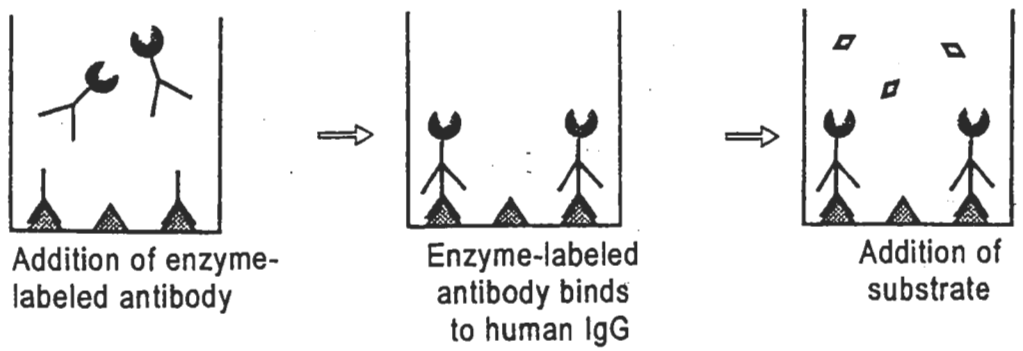
Human parvovirus B19 is known to cause conditions such as aplastic crisis in hemolytic anemia patients, chronic bone marrow failure in immunodeficiency patients, fifth disease in children, and various forms of arthritis in adults.[1) 2)] It has also been reported that human parvovirus B19 may cause fetal hydrops or stillbirth during pregnancy. Early diagnosis of human parvovirus B19 infection is necessary as it is a cause of a number of intractable diseases which directly impact human life. IgG-type antibody against human parvovirus B19 appears 1 to 2 weeks after infection and remains present over a long period of time. Consequently, the presence of IgG-type antibody indicates present or previous infection (anamnesis).

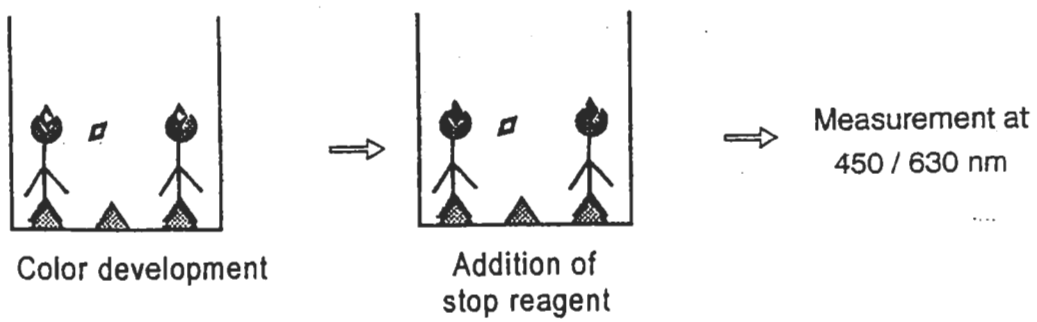
The PARVO IgG-EIA "SEIKEN" kit is an enzyme immunoassay for the detection of antibody to human parvovirus B19 in blood serum or plasma by employing virus empty particles produced by the baculovirus expression system with the same reactivity as native parvovirus B19 antigens fixed to a microplate.

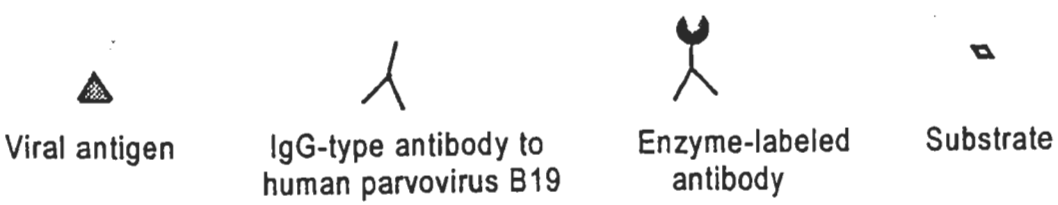
Use of the recombinant antigen ensures safety when utilizing the kit, and highly sensitive and specific detection of IgG-type antibody is possible.

PRINCIPLE OF TEST









FEATURES

1. Only a small sample volume (10 μL) is required.
2. Pretreatment of the sample is not necessary.
3. The test specifically detects IgG antibody.
4. The test can be completed within 3 hours, which includes time required for sample dilution and washing.
5. Allows for several samples to be tested simultaneously and economically, as separate microplate strips are used.

CONTENTS OF KIT

1. **Viral antigen-coated plate** **8 wells × 2 strips, 6 packs**
   Each well is coated with purified human parvovirus B19 recombinant antigen (3.0 μg/mL).
2. **Buffer solution** **250mL x 1**
   Phosphate-buffered saline containing 0.85 w/v% sodium chloride, 0.5 w/v% bovine serum albumin and 0.05 vol% Tween 20.
3. **Standard I** **2 mL x 1**
   Human serum containing little or no IgG-type antibody to human parvovirus B19. The serum has been diluted to an appropriate concentration for use and contains sodium azide as a preservative (0.1 w/v%).
4. **Standard II** **2 mL x 1**
   Human serum containing low-titer IgG-type antibody to human parvovirus B19. The serum has been diluted to an appropriate concentration for use and contains sodium azide as a preservative (0.1 w/v%).
5. **Standard III** **2 mL x 1**
   Human serum containing high-titer IgG-type antibody to human parvovirus B19. The serum has been diluted to an appropriate concentration for use and contains sodium azide as a preservative (0.1 w/v%).
6. **Enzyme-labeled antibody** (20 x conc.) **1 mL x 1**
   Phosphate-buffered saline containing peroxidase-labeled goat antibody against human IgG (20 μg/mL).
7. **Substrate** **20 mL x 1**
   Tetramethylbenzidine (0.3 mg/mL) solution containing 0.0075 vol% hydrogen peroxide.
8. **Stock wash solution** (10 x conc.) **100 mL x 1**
   Phosphate-buffered saline containing 8.5 w/v% sodium chloride and 0.5 vol% Tween 20.
9. **Stop reagent** **20 mL x 1**
   0.3 mol/L sulfuric acid.
10. **Plate holder** **1 pc.**

PRECAUTIONS

1. **General precautions**
   1) The kit should be used for *in vitro* diagnostic purposes only.
   2) Clinical symptoms and the results of other test methods should be considered in the diagnosis of human parvovirus B19.

2. Precautions for handling

1) The kit should be brought to room temperature (15 - 25 ℃) approximately 30 minutes before use.
2) The standards supplied with the kit should be included in each test run.
3) When adding specimens or the enzyme-labeled antibody solution to the wells, the pipette tip should not touch the well walls.
4) Incubation times should be performed exactly as indicated. Also, pipetting should be performed in order and in an even time interval between the wells.
5) Avoid rubbing, scratching, etc. the well walls.
6) To prevent contamination of specimens, pipette tips should be changed between specimens.

7) The following points should be noted when handling the substrate (TMB):
   (1) If the substrate is transferred to another container for use, use of the container with other reagents should be avoided.
   (2) Once transferred, the substrate should not be returned to the original vial.
   (3) Substrate which has turned blue during storage should not be used.
   (4) Operation in intense light should be avoided.
   (5) The plate should be kept away from light during the substrate incubation period.
   (6) The vial should be tightly recapped for storage immediately after use.
8) As the reagents in this kit contain sodium azide as preservative, flush with copious water to avoid the buildup of explosive metal azides.
9) As the stop reagent contains sulfuric acid, appropriate precautionary measures should be taken.
10) Although the standards supplied with the kit have been prepared from sera negative for HBs antigen, HIV antibody and HCV antibody, all standards and patient specimens should be treated as potentially infectious and handled with appropriate caution.
11) In the event that reagents contact with the skin, eyes or mouth, flush with plenty of water. Consulting a physician is recommended when it is considered necessary.
12) Do not freeze the reagents. Freezing and thawing the reagents might give erroneous test results.
13) Do not interchange caps among different reagents.
14) Do not use the reagent vials for other purposes after use.
15) Reagents with different lot numbers should not be interchanged or mixed for use. Mixing reagents from the same lot may also cause inaccurate results.
16) The test should be carried out according to the procedures in this kit insert, otherwise DENKA SEIKEN does not warrant the reliability of obtained data.

3. Precautions for disposal

1) The standards in this kit contain 0.1 w/v% sodium azide as a preservative. As sodium azide may react with lead or copper piping to form explosive metal azides, these reagents should be disposed of by flushing with copious amounts of water.
2) All specimens and equipment used in the test should be sterilized by one of the following methods after use:
   (1) Soaking in 2 vol% glutaraldehyde for 1 hour or longer.
   (2) Soaking in 0.5 w/v% sodium hypochlorite for 1 hour or longer.
   (3) Autoclaving at 121℃ for 20 minutes or longer.

TEST PROCEDURES

1. Materials which are necessary for the test but not provided:

1) Measuring pipettes 1 mL, 5 mL, 10 mL
2) Graduated cylinder
3) Ehlenmeyer flask (used forthepreparation of wash solution)

4) Small test tubes (used for the preparation of specimens)
5) Mixer for microtiter plate
6) Micropipettes 5 μL - 200 μL
7) Automatic plate reader (Spectrophotometer for microtiter plate)
   Plate reader equipped with dual wavelength system should be used for eliminating any potential interference caused by aberrations, such as dirt marks, on the optical surface of the well.
8) Distilled water

2. Preparation of reagents

Reagent	Preparation	Reagent name	Storage conditions
Viral antigen -coated plate	None		Upon opening, use plate immediately.
Buffer solution	None		
Standard I	None		
Standard II	None		
Standard III	None		
Enzyme-labeled antibody (20 x conc.)	According to the number of specimens to be tested, dilute an appropriate volume 20-fold with buffer solution.	Enzyme-labeled antibody solution	7 days 2-10℃ in a dark place.
Substrate	None		
Stock wash Solution (10 x conc.)	According to the number of specimens to be tested, dilute an appropriate volume of the stock wash solution 10-fold with distilled water.	Wash solution	Working solution should be used within the same day.
Stop reagent	None		

3. Preparation of specimens

Specimen	According to the number of specimen to be tested, prepare an appropriate number of small test tubes and add 2 mL of buffer solution to each test tube. Then distribute 10 μL of each specimen to the test tubes and mix well.

4. Test procedure

Remove an appropriate number of microtiter plate strips from the pack and place in the plate holder provided. Allot one well for a blank, each specimen, and Standards I and III, but use 2 wells for standard II (duplicate measurement).

<Step 1> Adding specimens and standards

1) Leaving the first well empty as blank, add 100 μL of each standard and pre-diluted specimen to their designated wells, keeping the order and time interval between additions constant.

2) Mix well by placing the plate on the plate mixer for several seconds. Cover the plate with aluminum foil and incubate at room temperature (15 – 25 ℃) for 1 hour in a vibration-free area.

<Step 2> Adding enzyme-labeled antibody solution

1) Remove the solutions from the wells by aspiration, keeping the order and time interval between aspirations constant.
2) Add approximately 200 μL of wash solution to each well and, after mixing for several seconds with a plate mixer, remove the solutions from the wells by aspiration. Repeat twice. Next, invert the plate and slap firmly on a stack of clean paper towels to remove any residual solution from the wells.
3) Add 100 μL of enzyme-labeled antibody solution to each well except for the blank, keeping the order and time interval between additions constant as in Step 1. Mix well by placing the plate on the plate mixer for several seconds. Cover the plate again with aluminum foil to protect from light and incubate at room temperature (15 – 25 ℃) for 1 hour in a vibration-free area.

<Step 3> Adding substrate

1) Remove the enzyme-labeled antibody solutions as in Step 2-1.
2) Repeat the wash procedure as in Step 2-2, washing a total of five times instead of three.
3) Add 100 μL of substrate to each well including the blank. Mix well by placing the plate on the plate mixer for several seconds. Cover the plate again with aluminum foil to protect from light and incubate at room temperature (15 - 25 ℃) for 30 minutes in a vibration-free area.

<Step 4> Adding stop reagent

1) Add 100 μL of stop reagent to each well including the blank, keeping the order and the time interval constant as in Step 1.
2) Within 1 hour of adding the stop reagent, measure the optical density (O.D.) of the wells at a filter wavelength of 450 / 630 nm with an automatic plate reader, after adjusting the instrument to zero-absorbance against the blank.

INTERPRETATION OF RESULTS

1. For the assay to be considered valid the following conditions must be met:

O.D. of Standard II	0.15 - 0.6
O.D. of Standard I / O.D. of Standard II	< 0.6
O.D. of Standard III / O.D. of Standard II	> 1.5
The ratio of the two O.D. values obtained for Standard II measurements should be less than 2 when the higher of the two values is the numerator.	

2. The O.D. value for the test specimen is expressed as "a", and the average of the O.D. values for Standard II is expressed as "b" as shown below.

3. Calculate the IgG antibody index for each specimen as follows:

IgG Antibody Index (x) = a / b

IgG Antibody Index(X)	Interpretation
X ≧1.00	Positive
X < 0.80	Negative
0.80 ≦ X < 1.00	Indeterminate

NOTES ON INTERPRETATION

1. It is useful to perform both the IgG-type antibody and IgM-type antibody assays simultaneously for an accurate assessment of the stage of human parvovirus B19 infection (i.e., non-, current or past infection). Generally, when both IgG and IgM are negative, the patient is considered free of infection. If a specimen shows a positive result in the IgM assay, a recent infection is indicated regardless of the results of the IgG assay. Finally, if a specimen gives a positive result in the IgG assay alone, a past infection is indicated.
2. If a specimen gives an indeterminate result, it is recommended that a second specimen be taken 1 - 2 weeks later for retesting or that another reference method such as PCR be tried. Clinical symptoms and the results of other test methods should be considered in the diagnosis of human parvovirus B19.

NOTES ON MEASUREMENT

1. Handling of specimen
   1) Use serum or plasma as specimen. Do not repeatedly freeze and thaw specimens.
   2) Do not heat inactivate or chemically treat specimens. If it is necessary to store specimens for later testing, specimens should be stored at -20℃ or lower. If heat inactivated specimens are used in the assay, erroneous test results may occur as shown below.

      [Influence of heat inactivation on test results]
      In an in-house study using 15 sera from patients who were confirmed to be suffering from erythema infectiosum and 38 sera from healthy subjects, changes in test results after heat inactivation were observed: one positive specimen turned indeterminate; one indeterminate specimen turned negative; 3 negative specimens turned indeterminate; and 3 negative specimens turned positive (Table 1).

2. Interfering substances
   1) The test is not significantly affected by anticoagulants such as heparin (up to 20 IU/mL), EDTA-2Na (up to 1 mg/mL) or citric acid (up to 3 mg/mL).
   2) The test is not significantly affected by endogenous substances like hemoglobin (up to 500 mg/dL), bilirubin (up to 30 mg/dL), ascorbic acid (up to 50 mg/dL) or intrafat (up to 5.0 %).

Table 1 Influence of heat inactivation on test results

Specimen	Before heat inactivation		After heat inactivation		Specimen	Before heat inactivation		After heat inactivation	
	Index	Interpr-etation	Index	Interpr-etation		Index	Interpr-etation	Index	Interpr-etation
1	7.76	+	8.25	+	25	0.48	−	0.62	−
2	3.81	+	5.01	+	26	0.45	−	0.89	±
3	4.53	+	5.29	+	27	0.70	−	0.52	−
4	5.69	+	6.72	+	28	0.45	−	0.59	−
5	3.63	+	4.83	+	29	0.59	−	0.51	−
6	6.48	+	8.29	+	30	0.33	−	0.41	−
7	3.92	+	4.53	+	31	0.18	−	0.24	−
8	7.31	+	6.05	+	32	0.32	−	0.49	−
9	5.55	+	5.18	+	33	0.41	−	0.53	−
10	7.23	+	6.24	+	34	0.23	−	0.34	−
11	10.75	+	2.70	+	35	0.81	±	0.48	−
12	5.27	+	9.86	+	36	0.56	−	0.53	−
13	3.00	+	3.91	+	37	0.58	−	0.67	−
14	10.85	+	10.93	+	38	0.72	−	0.77	−
15	1.36	+	1.63	+	39	0.49	−	0.73	−
16	0.64	−	1.41	+	40	0.25	−	0.53	−
17	0.26	−	0.47	−	41	0.70	−	0.43	−
18	0.40	−	1.48	+	42	0.95	±	0.99	±
19	0.19	−	0.50	−	43	0.60	−	0.90	±
20	0.24	−	0.38	−	44	0.58	−	0.44	−
21	0.22	−	1.02	+	45	0.61	−	0.41	−
22	0.32	−	0.65	−	46	0.60	−	0.34	−
23	0.37	−	0.62	−	47	1.12	+	0.80	±
24	0.41	−	0.82	±	48	0.82	±	0.86	±

NOTE: +; positive, - ;negative, ±; indeterminate. Specimens No. 1 to No. 15 are sera from patients confirmed to be suffering from erythema infectiosum and from No. 16 to 48 are sera from healthy subjects.

PERFORMANCE CHARACTERISTICS

When the test is performed according to "Test Procedure" (Section 4 of "Procedures" above), the following performance characteristics are observed.

1. Sensitivity

Positive test results were seen down to a 2^5 –fold dilution when control serum and plasma containing IgG-type antibody to human parvovirus B19 were assayed with this kit.

2. Specificity

Using five control sera and plasma that were respectively positive and negative for IgG-type antibody to human parvovirus B19, the test results were positive for positive samples, negative for negative samples, and negative for control sera.

3. Reproducibility

When tests were repeated five times using thre control sera and plasma that were positive for IgG-type antibody to human parvovirus B19, the plasma gave positive results only and the control sera gave negative results only.

STORAGE AND SHELF LIFE

Store at 2-10°C protected from light. Reagents are stable when stored under these conditions until the expiry date written on the box.

PACKAGE

PARVO IgG-EIA "SEIKEN" 96 tests/kit

REFERENCES

1) Cossart, Y. E., Field, A. M., Cant, B. & Widdows, D. : Parvovirus-like particles in human sera. Lancet i : 72-73, 1975.
2) Pattison, J. R., Jones, S. E., Hodgson, J., Davis, L. R., White, J. M., Stroud, C. E. & Murtaza, L. : Parvovirus infection and hypoplastic crisis in stckle cell anaemia. Lancet, i : 664, 1981.