Human Parvovirus B19 IgM antibody ELISA Kit

Enzyme Immunoassay for the qualitative screening of IgM class antibodies to Parvovirus B19 in serum or plasma.

Catalog number: ARG80597

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. Recombinant Parvovirus B19 antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
RF-IgG sorbent	6.5 ml (Ready-to-use)	4°C
Negative Control	2 ml (Ready-to-use)	4°C
Cut-Off Control	2 ml (Ready-to-use)	4°C
Positive Control	2 ml (Ready-to-use)	4°C
HRP-Antibody conjugate	20 ml (Ready-to-use)	4°C

20X Wash buffer	30 ml	4°C
Sample Diluent	100 ml (Ready-to-use)	4°C
TMB substrate	14 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450/620nm
- Pipettes and pipette tips
- Deionized or distilled water
- Vortex tube mixer
- 37°C incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- 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 two months if stored as described above.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved. Be sure that the crystals are

completely dissolved before use.

- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at 2-8°C for up to 5 days. For long-term storage samples should be aliquoted and stored at ≤ -20 °C and freeze-thaw only once. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at 2-8°C for up to 5 days. For long-term storage samples should be aliquoted and stored at ≤ -20 °C and freeze-thaw only once. Avoid repeated freeze-thaw cycles.

Note:

-Thawed samples should be inverted several times prior to testing.

-Do not use haemolytic, icteric or lipaemic specimens.

-Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. 10 mL + 190 mL) The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.
- Patient sample: Prior to assaying each patient specimen is first to be diluted with Sample Diluent. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with RF-IgG-Sorbent

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

  Note: the controls are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Leave 1 well for substrate blank, 1 well for the negative control, 2 wells for the Cut-off control and 1 well for the positive control.
3. Add 100 µl of diluted samples and controls into respective wells.
4. Cover the plate. Incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl HRP-Antibody conjugate solution into each well (expect the substrate blank). Incubate for 30 minutes at RT. In dark
7. Wash as according to step 5.
8. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
9. Add 100 µl of Stop Solution to each well. Any blue color developed during the incubation turns into yellow. Note: Highly positive patient samples can cause dark precipitates of the chromogen!
10. Read the OD with a microplate reader at 450 nm immediately. (620 nm as

optional reference wave length) and use the substrate controls as blank. The color is stable for at least 30 minutes.

CALCULATION OF RESULTS

1. Adjust the ELISA microplate or microstrip reader to zero using the substrate blank in well A1. If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
2. Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.
3. Dual wavelength reading using 620 nm as reference wavelength is recommended.
4. Where applicable calculate the mean absorbance values of all duplicates.

VALIDATION OF THE TEST RUN

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

Substrate blank: Absorbance value lower than 0.100

Neg. Control: Absorbance value lower than 0.200

Cut-off control (CO): Absorbance value between 0.350 – 0.850

Pos. Control: Absorbance value between 0.650 – 3.000

INTERPRETATION OF RESULTS

CO = Mean of OD of Cut-off value

POSITIVE: Patient (mean) absorbance values more than 20 % above CO (Mean OD patient > 1.2 x CO)

GREY ZONE: Patient (mean) absorbance values from 20 % above to 10 % below CO repeat test 2 - 4 weeks later with new patient samples (0.9 x CO ≤ Mean OD patient ≤ 1.2 x CO)

Results in the second test again in the grey zone ⇒ NEGATIVE

NEGATIVE: Patient (mean) absorbance values more than 10 % below CO (Mean OD patient < 0.9 x CO)

$$\frac{Patient\ (mean) absorbance\ value \times 10}{CO} = Unit;\ U$$

Cut-off value: 10 U

Grey zone: 9 - 12 U

Negative: < 9 U

Positive: > 12 U

QUALITY ASSURANCE

Assay Dynamic Range

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

Detection Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

Detection Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

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.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.14-6.24% and inter-assay precision was 6.29-14.68%.

Limitations of the Procedure

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