Parainfluenza virus Antibody IgA ELISA Kit

Cat. No.:DEIA1857
Pkg.Size:96T

Intended use

The Parainfluenza virus Antibody IgA ELISA Kit is intended for measurement of IgA class antibodies against Parainfluenza Virus IgA in human serum or plasma (citrate).

General Description

Parainfluenza viruses are important viral pathogens causing upper and lower respiratory infections in adults and children. The viruses belong to the family of Paramyxoviridae. They are enveloped single-stranded RNA viruses with spherical or pleomorphic shape. Parainfluenza viruses are relatively large viruses of about 150-300 nm in diameter. On the basis of antigenic differences they are divided into four subtypes, of which type 4 is divided into two subtypes. Parainfluenza virus 1 and 3 belong to the paramyxovirus genus. Parainfluenza virus 2, 4a and 4b belong to the rubella virus genus along with mumps. The virus is ubiquitous; infections occur as epidemics as well as sporadically. Parainfluenza viruses are sensitive to detergents and heat but can remain viable on surfaces for up to 10 hours. Transmission occurs via droplets, aerosols and fomites (viruses survive on surfaces). Parainfluenza virus infections are primarily childhood diseases, the highest age-specific attack rates for croup occur in children below the age of 3 years.

Principle Of The Test

The qualitative immunoenzymatic determination of IgA-class antibodies against Parainfluenza Viruses (Type 1, 2 and 3) is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microwells are precoated with Parainfluenza Virus antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgA conjugate is added. This conjugate binds to the captured Parainfluenza Virus-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Parainfluenza Virus-specific IgA antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

1. Parainfluenza Virus Coated Wells (IgA): 12 breakapart 8-well snap-off strips coated with Parainfluenza Virus antigen; in resealable aluminium foil.
2. IgA Sample Diluent ***: 1 bottle containing 100 mL of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
3. Stop Solution: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
4. Washing Solution (20x conc.)*:1 bottle containing 50 mL of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
5. Parainfluenza Virus anti-IgA Conjugate**:1 bottle containing 20 mL of peroxidase labelled rabbit antibody to human IgA; coloured violet, ready to use; black cap.
6. TMB Substrate Solution: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
7. Parainfluenza Virus IgA Positive Control***:1 bottle containing 2 mL; coloured yellow; ready to use; red cap.
8. Parainfluenza Virus IgA Cut-off Control***:1 bottle containing 3 mL; coloured yellow; ready to use; green cap.
9. Parainfluenza Virus IgA Negative Control***:1 bottle containing 2 mL; coloured yellow; ready to use; blue cap.

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* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

Materials Required But Not Supplied

1. ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
2. Incubator 37 °C
3. Manual or automatic equipment for rinsing wells
4. Pipettes to deliver volumes between 10 and 1000 µL
5. Vortex tube mixer
6. Deionised or (freshly) distilled water
7. Disposable tubes
8. Timer

Storage

The reagents are stable up to the expiry date stated on the label when stored at 2 °C - 8 °C.

Specimen Collection And Handling

Use human serum or plasma (citrate) samples with this assay.
If the assay is performed within 5 days after sample collection, the specimen should be kept at 2 °C - 8 °C; otherwise they should be aliquoted and stored deep-frozen (-20 °C to -70 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

Sample Dilution

Before assaying, all samples should be diluted 1+100with IgA Sample Diluent.
Dispense 10 µL sample and 1 mL IgA Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

Reagent Preparation

It is very important to bring all reagents, samples and controls to room temperature (20 °C – 25 °C) before starting the test run!

Coated snap-off Strips

The ready to use breakapart strips are coated with Parainfluenza Virus antigen. Store at 2 °C - 8 °C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 °C - 8 °C; stability until expiry date.

Parainfluenza Virus anti-IgA Conjugate

The bottle contains 20 mL of a solution with anti-human-IgA horseradish peroxidase, buffer, stabilizers, preservatives and an inert violet dye. The solution is ready to use. Store at 2 °C - 8 °C.
After first opening stability until expiry date when stored at 2 °C - 8 °C.

Controls

The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2 °C - 8 °C.
After first opening stability until expiry date when stored at 2 °C - 8 °C.

IgA Sample Diluent

The bottle contains 100 mL phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the sample specimen. This ready to use solution has to be stored at 2 °C - 8 °C.
After first opening stability until expiry date when stored at 2 °C - 8 °C.

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Washing Solution (20x conc.)

The bottle contains 50 mL of a concentrated buffer, detergents and preservatives.

Dilute washing solution 1+19; e.g. 10 mL washing solution + 190 mL fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. Crystals in the solution disappear by warming up to 37 °C in a water bath. After first opening the concentrate is stable until the expiry date.

TMB Substrate Solution

The bottle contains 15 mL of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2 °C - 8 °C, away from light. The solution should be colourless or have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discharged.

After first opening stability until expiry date when stored at 2 °C - 8 °C.

Stop Solution

The bottle contains 15 mL 0.2 M sulphuric acid solution (R 36/38, S 26), ready to use, store at 2 °C - 8 °C.

After first opening stability until expiry date.

Assay Steps

Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. 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 substrate blank,

1 well (e.g. B1) for the negative control,

2 wells (e.g. C1+D1) for the cut-off control and

1 well (e.g. E1) for the positive control.

It is recommended to determine controls and specimen samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100 µL controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour ± 5 min at 37±1°C.
4. When incubation has been completed, remove the foil, aspirate the content off the wells and wash each well three times with 300 µL of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

5. Dispense 100 µL Parainfluenza Virus anti IgA Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. Incubate for 30 min at room temperature. Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells
9. Incubate for exactly 15 min at room temperature in the dark.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.

Note: Highly positive specimen samples can cause dark precipitates of the chromogen! These precipitates have an influence

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when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in DU by 2.
11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

Measurement

Adjust the ELISA Microwell Plate Reader to zerousing 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!
Measure the absorbanceof all wells at 450 nmand record the absorbance values for each control and specimen sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable calculate the mean absorbance valuesof all duplicates.

Calculation

The cut-off is the mean absorbance value of the Cut-off control determinations.
Example: Absorbance value Cut-off control 0.44 + absorbance value Cut-off control 0.42 =0.86 / 2 = 0.43
Cut-off = 0.43

Interpretation of Results

Samples are considered POSITIVE if the absorbance value is higher than 10% over the cut-off.
Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative. → grey zone
It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered NEGATIVE.
Samples are considered NEGATIVE if the absorbance value is lower than 10% below the cut-off.

Precautions

1. All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
2. Do not interchange reagents or strips of different production lots.
3. No reagents of other manufacturers should be used along with reagents of this test kit.
4. Do not use reagents after expiry date stated on the label.
5. Use only clean pipette tips, dispensers, and lab ware.
6. Do not interchange screw caps of reagent vials to avoid cross-contamination.
7. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
8. After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
9. To avoid cross-contamination and falsely elevated results pipette specimen samples and dispense conjugate without splashing accuratelyto the bottom of wells.
10. The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

REFERENCES

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