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Human Poliomyelitis Virus IgG ELISA Kit

to detect Human Poliomyelitis Virus IgG in serum, plasma,

cell culture supernatants, cell lysates, tissue homogenates.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTuH9873DEA

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INTENDED USE

This kit is used to assay Human Poliomyelitis Virus IgG in Bovine serum, blood plasma, and other related tissue Liquid. *In vitro* use only.

This is a draft manual for reference purposes. Please always refer to the manual supplied with the shipment for any batch specific variations.

MANUAL VERSION 1.01

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PRINCIPLE

The kit uses a qualitative enzyme-linked immunosorbent assay (ELISA) to detect the presence of Human Poliomyelitis Virus IgG in samples. Samples containing Human Poliomyelitis Virus IgG are added to a pre-coated microwell followed by incubation and washing. Antibodies in the samples bind to the antigen on the plate. Unbound antibody is washed away during the washing step. A Horseradish Peroxidase (HRP) conjugated detection antibody is then added, incubated and washed. Finally, Chromogen Solution A and B are added which change color into blue, and to yellow in presence of an acid. By reading the absorbance of the solutions at 450nm wavelength and comparing it with the threshold value, the existence of the Poliomyelitis Virus IgG in the sample can be determined.

MATERIALS SUPPLIED IN THIS KIT

1	Positive control	0.5ml	7	Chromogen Solution A	6ml
2	Negative control	0.5ml	8	Chromogen Solution B	6ml
3	Coated Plate	12×8 strips	9	Stop Solution	6ml
4	HRP-Conjugate Reagent	6ml	10	Instruction Manual	1
5	30×wash solution	20ml	11	Plate Seal	2
6	Sample diluent	6ml	12	Zipper Bag	1

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator
2. Standard Enzyme reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

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IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8°C.
2. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
3. All samples and all discard generated should be disposed as per local rules.
4. Reagents of different batches must not be mixed and should be used before their respective validity dates.
5. Substrate B is sensitive to light and therefore should not be exposed to light for too long.
6. It is recommended that all standards, test samples are run at least in duplicates. If the testing material content in the sample is excessively high， then please use sample dilution solution to dilute the samples multiple-folds and multiply the Optical Density reading total dilution times accordingly at the time of calculation.

SAMPLE PREPARATION

1. Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).

2. After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

3. Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.

4. Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

5. Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

6. Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

7. Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add few mls of PBS (pH 7.4) and then homogenize the sample. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

8. CSF: It is recommended that freshly collected Cerebrospinal Fluid should be proceeded for immunological examination immediately without any storage or waiting for other microscopic/bacterial/chemical examinations. Fluid to be centrifuged at 1000-2000 RCF for 20 minutes, and supernatant should be used for further examination. In case of storage, centrifuge again prior to use.

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ASSAY PROCEDURE

1. Determine the number of strips required for the assay. Insert the strips in the Microplate frame for use. The unused strips should be stored at 4°C for up to one month.
2. Add 50µl negative control to each of the negative control wells and 50µl positive control to each of the positive control wells.
3. Dilute test samples five-folds with sample diluent, mix well and add to the micro-plate wells. The required 5-fold dilution is prepared by adding 10 µL sample to 40 µL of sample diluent.
4. Set the Blank wells without any solution and Incubate for 30 minutes at 37 ℃.
5. Dilute the washing concentrate (30X) with distilled water.
6. Remove the seal plate membrane carefully, drain the liquid in the wells and shake off the remaining liquid by tapping on an absorbent paper. Fill each well with washing solution. Drain the liquid after 60 seconds of soaking. Then repeat this procedure five times and blot the plate.
7. Add 50µl HRP-conjugate reagent to each well, except the blank well. Mix gently and incubate for 30 minutes at 37 ℃.
8. Drain the liquid in the wells and shake off the remaining liquid by tapping on an absorbent paper. Fill each well with washing solution. Drain the liquid after 60 seconds of soaking. Then repeat this procedure five times and blot the plate.

9. Add 50µl chromogen solution A firstly to each well. Then add 50µl chromogen solution B to each well. Shake gently to mix the wells and incubate for 10 minutes at 37℃ away from light for color development.
10. Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).
11. Set blank well as zero absorbance and measure the absorbance (OD) of each well at 450nm wavelength using a microplate reader. This reading should be taken within 10 minutes after having added the stop solution.

RESULT DETERMINATION

Test validity: the mean of Positive control wells ≥ 1.00 and the mean of Negative control wells < 1.00.

Calculate Threshold: Threshold = (the mean of Negative control wells) + 0.15

If the mean of Negative Controls is less than 0.05, then use threshold as 0.20

Test Sample Status Determination:

Sample Status Negative: If Test Sample OD< Threshold.

Sample Result Positive: If Test Sample OD≥ Threshold

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PROTOCOL SUMMARY

Prepare samples and reagents.

Add prepared samples and controls in respective wells and Incubate for 30 minutes at 37℃.

Wash the plate five times. Add HRP-Conjugate Reagent and incubate for 30 minutes at 37℃.

Wash the plate five times. Add Chromogen Solution A and B. Incubate for 10 minutes at 37 ℃ for color development.

Add stop solution

Read the OD value within 10minutes.

Calculate and determine result.

PACKAGE SIZE：96Tests.

VALIDITY & STORAGE: Six months (at 2-8°C) or Twelve months (at -20°C) from date of purchase. Once opened, kit to be consumed in one month. Avoid repeated freeze thaw cycles.