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Human Influenza Viruses B (FLU B) ELISA

REF : KBVH046

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Influenza Viruses B (FLU B) in human serum and plasma

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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REF **KBVH046** **Σ 96 tests**

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Introduction:

Influenza B virus is the only species in the genus Betainfluenzavirus in the virus family Orthomyxoviridae. Influenza B virus is only known to infect humans and seals with influenza. This limited host range is apparently responsible for the lack of associated influenza pandemics in contrast with those caused by the morphologically similar influenza A virus as both mutate by both antigenic drift and reassortment. There are two known circulating lineages of Influenza B virus based on the antigenic properties of the surface glycoprotein hemagglutinin.

Intended Use:

Human Influenza Viruses B ELISA is used as an analytical tool for quantitative determination of FLU B ELISA in human serum, plasma and cell culture supernatant.

Principle:

This is sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human FLU B in samples. Addition of standard or sample to microtiter well which is pre-coated with Human FLU B monoclonal antibody and addition of biotin labeled Human FLU B antibody, followed by addition of HRP conjugate to form immune complex. Unbound HRP conjugate will get removed by washing step after incubation. Then addition of Substrate A and B, develops blue color during incubation period and reaction will get stop after addition of stop solution with development of yellow color. The concentration of the Human Influenza Viruses B of sample is directly proportional to the yellow color developed in well and will be positively correlated.

Materials Provided:

1. Microtiter Coated Plate (96 wells) - 1 no
2. Biotinylated Anti-FLU B Detection Antibody - 1 ml
3. Standard (4000 pg/ml) - 0.5 ml
4. Streptavidin:HRP Conjugate - 6 ml
5. (30X) Wash Buffer - 20 ml
6. Standard Diluent - 3 ml
7. TMB Substrate A - 6 ml
8. TMB Substrate B - 6 ml
9. Stop Solution - 6 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Semi-Log graph paper or software for data analysis
6. Tubes to prepare standard /sample dilutions
7. Timer
8. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources

Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make 1X Wash Solution, add 10ml of 30X Wash Buffer in 290ml of DI water.
3. Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and standard diluent.

Standard Concentration ng/ml	Standard No	Standard Particulars
4000 pg/ml	Standard No.8	Original Standard
2000 pg/ml	Standard No.7	120 ul Original Standard + 120 ul Standard diluents
1000 pg/ml	Standard No.6	120 ul Standard No.6 + 120 ul Standard diluents
500 pg/ml	Standard No.5	120 ul Standard No.5 + 120µl Standard diluent
250 pg/ml	Standard No.4	120 ul Standard No.4 + 120 ul Standard diluent
125 pg/ml	Standard No.3	120 ul Standard No.3 + 120 ul Standard diluent
62.5 pg/ml	Standard No.2	120 ul Standard No.2 + 120 ul Standard diluent
0 pg/ml	Standard No.1	120 ul Standard No.1 + 120 ul Standard diluent

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the antibodies.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.

2. Pipette out **50 µl of Standards and 40 µl Samples** into the respective wells as mentioned in the work list. *Note: do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.*

3. Pipette out **10 µl of Biotin Conjugate** into each sample well.
Do not pipette into the blank and standards wells.

4. Pipette out **50 µl of Streptavidin-HRP Conjugate** into each sample and standards well.
Do not pipette into the Blank well.

5. Cover the plate and incubate for 1 hour at 37°C in the incubator.

6. Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

7. Add **TMB Substrate A 50 ul**, then **TMB Substrate B 50 ul** to each well including Blank well; mix gently.

8. Incubate in the dark at 37ºC in for 10-30 minutes (Do not exceed 30mins) and observe the wells every 5 mins time interval. Positive wells should turn bluish in color.

9. Pipette out **50 ul of Stop Solution**. Wells should turn from blue to yellow in colour.

10. Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution blanking on the zero standards.

Calculation of Results:

Take the standard density as the horizontal axis, OD values in the vertical axis and draw the standard curve on graph paper. Find out the corresponding concentration according to the sample OD value from the sample curve (the result is the sample concentration) or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be less than **62.5 pg/ml.**

Assay range:
62.5 pg/ml - 4000 pg/ml

Precision:
Intra-assay Precision: 3 samples with low, middle and high level Human Influenza Viruses B were tested 20 times on one plate, respectively.
Inter-assay Precision: 3 samples with low, middle and high level Human Influenza Viruses B were tested on 3 different plates, 8 replicates in each plate.
CV (%) = SD/mean x 100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Safety Precautions:

- **This kit is for research use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

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