Human Complement component C6, C6 GENLISA™ ELISA

REF : KBH5098

Ver 2.0

RUO

Enzyme Immunoassay for the Quantitative determination of Human Complement component C6, C6 in serum, plasma and cell culture supernatant

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

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REF KBH5098 Σ 96 tests

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Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The Human Complement component C6, C6 GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human Complement component C6, C6 in serum, plasma and cell culture supernatant.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Complement component C6, C6 present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin-HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substHumane solution (TMB) is added to microwells and color develops proportionally to the amount of Human Complement component C6, C6 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. C6 Antibody Coated Microtiter Plate (12 x 8 wells) – 1 no
2. ConcentHumaned Standard, Human C6 (800 µg/ml) – 0.5 ml
3. Biotinylated C6 Antibody – 1 ml
4. Streptavidin-HRP Conjugate – 6 ml
5. Standard Diluent – 3 ml
6. 25X Wash Buffer – 20 ml
7. SubstHumane A – 6 ml
8. SubstHumane B – 6 ml
9. Stop Solution – 6 ml

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 25µl to 1000µl
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. 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 tempeHumanure (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The SubstHumane is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

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

Sample PrepaHumanion and Storage:

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

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room tempeHumanure for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citHumane plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentHumanion is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

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

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

1. Label any aliquots made with the kit Lot No and ExpiHumanion date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room tempeHumanure before use.
3. To make **Wash Buffer (1X)**; dilute **20 ml of 25X Wash Buffer in 480 ml of DI water**.
4. **Standards PrepaHumanion**: Dilute 120 µl of original **Standard (800 µg/ml)** with 120 ul of standard diluent to geneHumane a **400 µg/ml Standard stock solution**. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the **Standards** by serially diluting the standard stock solution as per the below table.

Standard ConcentHumani	Standard Vial	Dilution Particulars
400 µg/ml	Standard No.5	120 ul Standard Provided (800 µg/ml) + 120 ul Standard Diluent
200 µg/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
100 µg/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
50 µg/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
25 µg/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentHumanions of Human Complement component C6, C6. High Dose Hook Effect is due to excess of antibody for very high concentHumanions of Human Complement component C6, C6 present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
3. Human Complement component C6, C6 concentHumanion of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Complement component C6, C6.
5. It is recommended that all Standards and Samples be assayed in duplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the SubstHumane has a distinct blue color prior to use it may have been contaminated and use of such substHumane can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50 ul Standard** to standard well. *Note: Do not add* **Biotinylated C6 Antibody** *to standard well because the Standard Solution contains the biotinylated antibody.*

3. Add **40 ul Sample** to respective sample wells.

4. Pipette **10 ul Biotinylated C6 Antibody** to respective sample wells.

5. Pipette **50 ul Streptavidin-HRP Conjugate** to respective sample wells and also the standard wells. *Note: Do not add the* ***Strepatividin-HRP Conjugate*** *to the blank well*.

6. Mix well. Cover the plate with a sealer and incubate for **60 minutes** at **37°C.**

7. AspiHumane and wash plate 4 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

8. Pipette **50 ul SubstHumane A** followed by **50 ul SubstHumane B** in all the wells.

9. Incubate the plate at at 37°C for 10 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette **50 ul** of **Stop Solution** in all wells. The wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentHumanion of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human Complement component C6, C6 concentHumanions, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Human Complement component C6, C6 ConcentHumanion.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to geneHumane a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Quality Control:

It is recommended that for each laboHumanory 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:

This kit has been validated. Please view the details herein below.

Standard CalibHumanor Provided:
ConcentHumanion - 800 µg/ml

Standard CalibHumanion Range:
25 µg/ml – 400 µg/ml

Limit Of Detection:
It is defined as the lowest detectable concentHumanion 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 1.17 µg/ml.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiHumanion 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 prepaHumanion 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.

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