GENLISA® Human Cystatin 3, CST-3 ELISA

REF: KBH9656

Ver 1.1

PackSize: 96 wells

For Research Use Only

Enzyme Immunoassay for the Quantitative Determination of Cystatin 3, CST-3 in Human serum, plasma and other biological samples

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 KBH9656 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 GENLISA® Human Cystatin 3, CST-3 ELISA kit is used as an analytical tool for quantitative determination of Human Cystatin 3, CST-3 in serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Cystatin 3, CST-3 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 substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Cystatin 3, CST-3 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Human Cystatin 3 Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Human Cystatin 3 Standard (lyophilized, concentrated, 200 ng/ml) - 2 vials
3. Biotinylated Cystatin 3 Antibody (concentrated) - 120 ul
4. Streptavidin:HRP Conjugate (concentrated) - 120 ul
5. Standard Diluent - 20 ml
6. Biotin Antibody Dilution Buffer - 12 ml
7. HRP Conjugate Dilution Buffer - 12 ml
8. (25X) Wash Buffer - 20 ml
9. TMB Substrate - 12 ml
10. Stop Solution - 12 ml
11. 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. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator
8. Timer.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
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.

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 Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate 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 temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate 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. **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.
5. **Cell Culture supernatants and other biological fluids-** Centrifuge samples at 1000 x g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20˚C or -80˚C for later use. Avoid repeated freeze-thaw cycles.

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

Sample Dilution

Please note the kit is validated for use with neat samples.
Incase the user wishes to estimate the concentration of the target protein in the test sample, we advise to select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit and several trials may be necessary. If samples contain very high concentrations of the analyte, dilute the samples with the Standard Diluent provided in the kit.

Please refer to the following table of recommended dilution for samples:

1. For 50 fold dilution: One-step dilution. Add 5 ul sample to 245 ul standard/sample diluent to yield 50 fold dilution.
2. For 100 fold dilution: One-step dilution. Add 5 ul sample to 495 ul standard/sample diluent to yield 100 fold dilution.
3. For 1000 fold dilution: Two-step dilution. Add 5 ul sample to 95 ul standard/sample diluent to yield 20 fold dilution, then add 5 ul of 20 fold diluted sample to 245 ul standard/sample diluent to yield 50 fold dilution. At last sample diluted 1000 fold.
4. For 100000 fold dilution: Three-step solution. Add 5 ul sample to 195 ul standard/sample diluent to yield 40 fold dilution, then add 5 ul of 40 fold diluted sample to 245 ul standard/sample diluent to yield 50 fold dilution, finally add 5 ul of 2000 diluted sample to 245 ul standard/sample diluent to yield 50 fold dilution. At last sample diluted 100000 fold.

Notes:

1. *Take at least 3 ul standard/sample dilution at each dilution steps. Dilution ratio should not exceed 100 times. Too small sample volume is easy to cause greater errors during the mixing process. Each dilution step needs to be mixed evenly to avoid foaming.*
2. *If the dilution ratio is very high, dilute it with PBS first, then use standard/sample diluent at the last dilution step.*

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

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.

2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X) 500 ml;** dilute **20 ml of (25X) Wash Buffer in 480 ml of DI water**.
4. **Streptavidin:HRP Conjugate & Biotinylated Cystatin 3 Antibody Working Solution** - Briefly spin or centrifuge the Streptavidin:HRP Conjugate & Biotinylated Cystatin 3 Antibody before use. Dilute them to the working concentration 100-fold with HRP Conjugate Dilution Buffer & Biotin Antibody Dilution Buffer, respectively.
5. **Standards Preparation**: Reconstitute original Cystatin 3, CST-3 with 1.0 ml of Standard Diluent. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
200 ng/ml	Standard No.8	Reconstitute with 1.0 ml Standard Diluent
100 ng/ml	Standard No.7	500 ul Standard No.8 + 500 ul Standard Diluent
50 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
25 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
12.5 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
6.25 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
3.13 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
0 ng/ml	Standard No.1	500 ul Standard Diluent only

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 concentrations of Human Cystatin 3, CST-3. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Cystatin 3, CST-3 present in the sample.
3. Human Cystatin 3, CST-3 concentration 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 Cystatin 3, CST-3.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
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 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.
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 **100 ul Standard Diluent** to blank wells.

3. Add **100 ul prepared Standards and Samples** to respective wells.

4. Cover the plate with a sealer and incubate for 80 minutes at 37°C.

5. Aspirate 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.

6. Pipette **100 ul Biotinylated Cystatin 3 Antibody Working Solution** to all wells.

7. Cover the plate with a sealer and incubate for 50 minutes at 37°C.

8. Aspirate and wash as per Step (5) above.

9. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.

10. Cover the plate with a sealer and incubate for 50 minutes at 37°C.

11. Aspirate and wash as per Step (5) above.

12. Pipette **100 ul TMB Substrate** in all the wells.

13. Incubate the plate at **37°C** for **20 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

14. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

15. 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 concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human Cystatin 3, CST-3 concentrations, 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 Cystatin 3, CST-3 Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL 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 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:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
3.13 ng/ml – 200 ng/ml

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 2.47 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of Cystatin 3, CST-3. No significant cross-reactivity or interference between Cystatin 3, CST-3 and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of Cystatin 3, CST-3 and the recovery rates were calculated by comparing the measured value to the expected amount of Cystatin 3, CST-3 in samples.

Matrix	Recovery Range (%)	Average (%)
serum(n=5)	79-91	85
EDTA plasma(n=5)	81-97	89
heparin plasma(n=5)	84-99	91

Precision:
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Cystatin 3, CST-3 and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	85-92%	88-96%	87-98%	95-102%
EDTA plasma(n=5)	98-105%	96-103%	87-95%	83-92%
heparin plasma(n=5)	95-104%	87-96%	89-101%	97-105%

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.
- 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.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.1 Standard No.1			
1B 2B	Standard No.2 Standard No.2			
1C 2C	Standard No.3 Standard No.3			
1D 2D	Standard No.4 Standard No.4			
1E 2E	Standard No.5 Standard No.5			
1F 2F	Standard No.6 Standard No.6			
1G 2G	Standard No.7 Standard No.7			
1H 2H	Standard No.8 Standard No.8			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	Coated Microtiter Plate (12 x 8 wells)
STD	Standard
BIOTIN | AB	Biotinylated Antibody
HRP | CONJ	Conjugate Horseradish Peroxidase
BIOTIN | DIL	Biotin Antibody Dilution Buffer
HRP | DIL	HRP Conjugate Dilution Buffer
STD | DIL	Standard Diluent
25X | WASH | BUF	(25X) Wash Buffer
SUB | TMB	TMB Substrate
SOLN | STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature