






# GENLISA™ Human Cluster of Differentiation 146, sCD146 ELISA

**REF** : KBH0309

Ver 1.1


**RUO**


Enzyme Immunoassay for the quantitative determination of human Cluster of Differentiation 146, sCD146 in serum, plasma and cell culture supernatant

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

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**REF** KBH0309

 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.

CD146/sCD146 is a cell adhesion molecule expressed on endothelial cells, as well as on other cells such as mesenchymal stem cells and Th17 lymphocytes. This protein also exists in a soluble form, whereby it can be detected in biological fluids, including the serum or the cerebrospinal fluid (CSF). Some studies have highlighted the significance of CD146 and its soluble form in angiogenesis and inflammation, having been shown to contribute to the pathogenesis of many inflammatory autoimmune diseases, such as systemic sclerosis, mellitus diabetes, rheumatoid arthritis, inflammatory bowel diseases, and multiple sclerosis.

**Intended Use:**

The GENLISA™ Human Cluster of Differentiation 146, sCD146 ELISA kit is used as an analytical tool for quantitative determination of Human Cluster of Differentiation 146, sCD146 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 Cluster of Differentiation 146,sCD146 present in The samples are bound by the antibodies. HRP (horseradish peroxidase) conjugated secondary antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Cluster of Differentiation 146,sCD146 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

1. Human Cluster of Differentiation 146, sCD146 Antibody Coated Microtiter Plate (12 x 8 wells) -1 no
2. Human Cluster of Differentiation 146, sCD146 Standard (lyophilized, concentrated 40 ng/ml) - 1 vial
3. Anti-sCD146:HRP Conjugate - 12ml
4. Standard Diluent - 10 ml
5. Sample Diluent - 50 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. 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. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

**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 (18-25°C). 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. **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 concentration 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.

**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)**; dilute **25 ml of 20X Wash Buffer** in **475 ml** of DI water.
4. **Standards Preparation:** Reconstitute lyophilized standard with 100ul of Standard Diluent to get a concentration of 40 ng/ml. Keep the standard for 15 mins with gentle agitation before making further dilutions. Dilute 70 ul of reconstituted standard with 430 ul of Standard diluent to get a concentration of 5600 pg/ml. Standard range for Human Cluster of Differentiation 146,sCD146 is 5600 pg/ml, 2800 pg/ml, 1400 pg/ml, 700 pg/ml, 350 pg/ml, 175 pg/ml and 87.5 pg/ml . Standard Diluent is used as “zero” standard

Standard Concentration	Standard Vial	Dilution Particulars
40 ng/ml	lyophilized, Concentrated	Lyophilized standard + 100 ul Standard Diluent
5600pg/ml	Standard No.7	70 ul Reconstituted Standard + 430 ul Standard Diluent
2800pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent
1400 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent
700 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard Diluent
350 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent
175 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent
87.5 pg/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard Diluent
0 pg/ml	Standard No.0	Only 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 concentrations of Human Cluster of Differentiation 146, sCD146.

High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Cluster of Differentiation 146, sCD146 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 Cluster of Differentiation 146, sCD146 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 ( $\text{NaN}_3$ ), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Cluster of Differentiation 146, sCD146.
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 compromise 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** and **Sample** and incubate at Room Temperature for 2 hours.
3. 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.
4. Add **100 ul Anti-sCD146:HRP Conjugate** and incubate at Room Temperature for 1 hour.
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 TMB Substrate** to all wells and incubate at Room Temperature for 30 minutes.
7. Pipette **100 ul of Stop Solution** in all wells. The wells should turn from blue to yellow in color.
8. Read the absorbance at 450 nm with a microplate within 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 Cluster of Differentiation 146, sCD146 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 Cluster of Differentiation 146, sCD146.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a 4PL (2<sup>nd</sup> order) or 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.

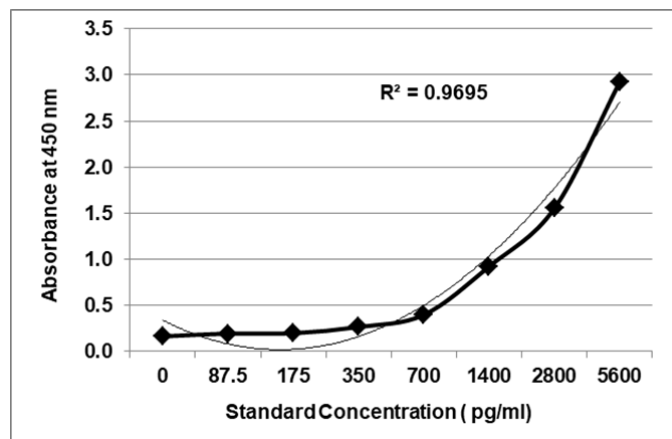
#### **Quality Control:**

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

**Typical Data**

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.164	--	-
87.5	0.194	99.7	113.9
175	0.198	168.6	96.4
350	0.265	311.9	89.1
700	0.398	576.5	82.4
1400	0.923	1555.5	111.1
2800	1.552	2734.1	97.6
5600	2.922	5611.3	100.2

**Typical Graph**



**Performance Characteristics of the Kit:**

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

**Standard Calibration Range:**

87.5 pg/ml – 5600 pg/ml

**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 80 pg/ml.

**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 ~85 pg/ml.

**Specificity:**

The antibodies used in this kit are monoclonal antibodies specific for Human Cluster of Differentiation 146, sCD146. The standard used in the kit is a recombinant protein expressed in HEK293 cells using a DNA sequence encoding the extracellular domain of human CD146 precursor (NP\_006491.2) (Met 1-Gly 559).

**Precision:**

Intra-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Human sCD146 were tested in triplicate respectively. The Intra-Assay was found to be <10%

Inter-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Human sCD146 were tested in triplicate on two plates respectively on two consecutive days. The Inter-Assay was found to be <12%.

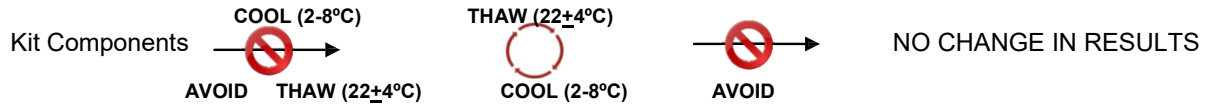
*The Cumulative Variance % was calculated as CV (%) = SD/mean x 100 [SD=standard deviation]*

**SCHEMATIC ASSAY PROCEDURE**

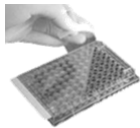

1. Remove all components, 30 minutes before adding into the assay plate.




2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

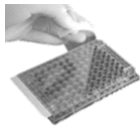




3.  Pipette **100 ul prepared Standards / Samples** into respective wells.

4.  Cover plate and incubate for  at room temperature.

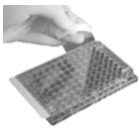

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X)**.

6.  Pipette **100 ul Anti- sCD146:HRP Conjugate** to all wells.

7.  Cover plate and incubate for  at room temperature.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X)**.

9.  Pipette **100 ul TMB Substrate** to all wells.

10.  Cover plate and incubate for  at room temperature.

11.  Pipette **100 ul Stop Solution** in all wells.

12. Read absorbance at 450nm with a  microplate reader within  of stopping reaction.

**Typical Example of a Work List**

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.0 Standard No.0			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Standard No.6 Standard No.6			
1H 2H	Standard No.7 Standard No.7			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

**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.

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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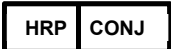



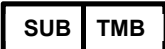





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**SYMBOLS KEY**

	Human Cluster of Differentiation 146, sCD146 Coated Microtiter Plate (12x8 wells)
	Standard lyophilized
	Conjugate Horseradish Peroxidase
	Standard Diluent
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature