# KRIBIOLISA™ Sunitinib (SUTENT) ELISA

**REF** : KOD1019

Ver1.1

**RUO**

Enzyme Immunoassay for the quantitative determination of Sunitinib in serum, plasma and cell culture supernatant

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



**REF KOD1019** **Σ 96 tests**

**KRISHGEN BioSystems** Unit Nos#318/319, Shah & Nahar,
Off Dr E Moses Road, Worli, Mumbai 400018.
Tel: 91 (22) 49198700 | Email: sales@krishgen.com
http://www.krishgen.com

**Introduction:**

Sunitinib (SUTENT) is a medication used to treat cancer. It is a small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor that was approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST). Sunitinib Malate is an antineoplastic agent, Flk-1 inhibitor, and ATP-competitive inhibitor of PDGFRβ.

**Intended Use:**

This kit is used for quantitative estimation of Sunitinib in serum, plasma and cell culture supernatant using a competitive inhibition principle.

**Principle:**

The KRIBIOLISA™ Sunitinib ELISA is a competitive inhibition immunoassay for the determination of Sunitinib. The microplate is coated with VEGFA protein. VEGFA protein is a known dimeric glycoprotein which functions as a ligand for the receptor VEGFR2 (Flk1). Samples and standards containing Sunitinib is added to the plate with HRP conjugated VEGFR-2 from the kit simultaneously. The Sunitinib in the samples and standard will inhibit the binding of VEGFR-2 to the microplate. The amount of Sunitinib is inversely proportional to the color developed in the microplate post addition of TMB substrate and stopping the reaction. The absorbance is read at 450 nm.

**Materials Provided:**

1. VEGFA coated Microtiter Plate (12x8 wells) - 1 no
2. Sunitinib Standard (concentrated, 1mg) - 1vial
3. VEGFR-2:HRP Conjugate - 12 ml
4. (20X) Wash Buffer - 25 ml
5. Assay Diluent - 50 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12ml
8. Instruction Manual

**Materials to be provided by the End-User:**

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

**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.

**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:**

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

**Preparation Before Use:**

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

**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. Standard: The concentration of the standard in the stock solution is 1mg/ml. Prepare the standards as per the table given below using the provided standard Concentration and standard diluent. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 500, 250, 125, 62.5, 31,25, 15.526 and 0 ug/ml

| Standard Concentration | Standard No. | Dilution Particulars |
|---|---|---|
| 1000 ug/ml | Original Standard | Original Standard concentrated provided |
| 500 ug/ml | Standard No.7 | 100 ul original standard provided + 100 ul Assay Diluent |
| 250 ug/ml | Standard No.6 | 100 ul Standard No.7 + 100 ul Assay Diluent |
| 125 ug/ml | Standard No.5 | 100 ul Standard No.6 + 100 ul Assay Diluent |
| 62.5 ug/ml | Standard No.4 | 100 ul Standard No.5 + 100 ul Assay Diluent |
| 31.25 ug/ml | Standard No.3 | 100 ul Standard No.4 + 100 ul Assay Diluent |
| 15.625 ug/ml | Standard No.2 | 100 ul Standard No.3 + 100 ul Assay Diluent |
| 0 ug/ml | Standard No.1 | 100 ul Assay 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. 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.
9. Making serial dilution in the wells directly is not permitted.
10. **Prepare standard within 15 minutes before assay.** Please do not dissolve the reagents at 37ºC directly.
11. **HRP Conjugate** is a sticky solution; therefore, slowly pipette them to reduce the volume errors.
12. Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettes are calibrated. It is recommended to suck more than 10µL for one pipetting.

13. If crystals have formed in the Wash Solution concentrate (20X), warm to room temperature and mix gently until the crystals are completely dissolved.
14. Contaminated water or container for reagent preparation will influence the detection result.

**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. All steps must be performed at 37°C.

2. Pipette out **100 ul of Sunitinib Standards** and **Samples** to respective wells.

3. Add **100 ul of HRP conjugated VEGFR-2** to all wells.

4. Cover the plate and incubate for 120 minutes at 37°C.

5. Aspirate and wash plate 4 times with **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. Add **100 ul** of **TMB Substrate** in each well.

7. Incubate the plate at 37°C for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

8. Pipette out **100 ul** of **Stop Solution** into all wells. Wells should turn from blue to yellow in color.

9. Read the absorbance at 450 nm with a microplate reader.

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

**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:**

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:**
The minimum detectable dose of Sunitinib is 14 ng/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by subtracting two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

**Precision:**
Intra-assay Precision: 3 samples with low, middle and high level human Sunitinib were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level human Sunitinib 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.

**References:**

Determination of sunitinib and its active metabolite, N-desethyl sunitinib in mouse plasma and tissues by UPLC-MS/MS: assay development and application to …
X Chen, Z Wang, M Liu, M Liao, X Wang… - Biomedical …, 2015 - Wiley Online Library

Pharmacokinetics and safety of sunitinib malate in subjects with impaired renal function
R Khosravan, M Toh, M Garrett… - The Journal of …, 2010 - Wiley Online Library

Sunitinib malate is active against human urothelial carcinoma and enhances the activity of cisplatin in a preclinical model
G Sonpavde, W Jian, H Liu, MF Wu, SS Shen… - … Oncology: Seminars and …, 2009 - Elsevier

Study of the cellular mechanism of Sunitinib mediated inactivation of activated hepatic stellate cells and its implications in angiogenesis
S Majumder, AC Piguet, JF Dufour… - European journal of …, 2013 - Elsevier

## Typical Example of a Work List

| Well # | Contents | Absorbance at 450nm | Mean Absorbance | ng/ml Sunitinib equivalent |
|---|---|---|---|---|
| 1A<br>2A | zero std<br>zero std | | | |
| 1B<br>2B | Standard 1<br>Standard 1 | | | |
| 1C<br>2C | Standard 2<br>Standard 2 | | | |
| 1D<br>2D | Standard 3<br>Standard 3 | | | |
| 1E<br>2E | Standard 4<br>Standard 4 | | | |
| 1F<br>2F | Standard 5<br>Standard 5 | | | |
| 1G<br>2G | Standard 6<br>Standard 6 | | | |
| 1H<br>2H | Standard 7<br>Standard 7 | | | |
| 3A<br>4A | Sample | | | |
| 3B<br>4B | Sample | | | |

**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 product; 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 product 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 product 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, 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.

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



***THANK YOU FOR USING KRISHGEN PRODUCT!***