KRIBIOLISA™ Octreotide (Sandostatin) ELISA

REF : KBI5024

Ver 2.1

RUO

Immunoassay for Quantitative Estimation of Octreotide 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

REF KBI5024 Σ 96 tests

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Introduction:

Octreotide (Sandostatin) is an octapeptide that mimics natural somatostatin pharmacologically, though it is a more potent inhibitor of growth hormone, glucagon, and insulin than the natural hormone.

Intended Use:

The KRIBIOLISA™ Octreotide ELISA is used for estimation of Octreotide in serum and plasma in pharmacokinetics, peptide delivery study and other purposes.

Principle:

The KRIBIOLISA™ Octreotide ELISA is a competitive immunoassay for the determination of Octreotide. Anti-Octreotide antibodies are coated on microplates and a constant concentration of Octreotide:HRP Conjugate and varying concentration of unlabeled Octreotide Standard or in the sample compete for binding to the Anti-Octreotide antibodies. Captured HRP conjugated Octreotide produces a soluble colored product after addition of TMB substrate. The enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is inversely proportional to the amount of bound Octreotide molecule present in standards or samples.

Materials Provided:

Part	Description	Qty
Anti-Octreotide Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Octreotide antibody.	1 x 96 wells
Octreotide Standard	Recombinant Octreotide standard – (lyophilized ; 1 ug/ml)	2 vials
Octreotide:HRP conjugate	HRP Conjugated Octreotide prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and 1:1000 human serum and preservative thiomersol < 0.01%	10 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer and preservative thiomersol < 0.01%	2 x 50 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

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.

Storage Information:

1. Store kit components at 2-8°C.

2. All the reagents and wash solutions should be used within 12 months from manufacturing date. Before using, bring all components to room temperature (18-25ºC).
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

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 Serum & Plasma - Samples have to be diluted 1:1000 (v/v), e.g. 1 ul sample + 999 ul (1X) Sample Diluent prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Note:
1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

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

1. Bring all kit components and samples to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. To make **Wash Buffer (1X)**; dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.
3. **Standard:** Reconstitute the lyophilized standard in 1000ul of Standard diluent to get a concentration of 1000 ng/ml. Keep the standard for 15 minutes. Add 640ul of 1 ug/ml standard to 360ul of Standard Diluent to get 640 ng/ml. Standard range for Octreotide ELISA is 640 ng/ml, 320 ng/ml, 160 ng/ml, 80 ng/ml, 40 ng/ml, 20ng/ml and 10 ng/ml. Standard Diluent (1X) serves as the zero standard (0 ng/ml).

Standard Concentration	Standard No	Dilution Particulars
1000 ng/ml	Reconstituted standard	Lyophilized Standard provided in the Kit + 1000ul of Standard Diluent (1X).
640 ng/ml	Standard No. 7	640ul of Reconstituted standard + 360 ul of Standard Diluent (1X)
320 ng/ml	Standard No.6	500 ul of Standard No. 7 + 500 ul Standard Diluent (1X)
160 ng/ml	Standard No.5	500 ul of Standard No. 6 + 500 ul Standard Diluent (1X)
80 ng/ml	Standard No.4	500 ul of Standard No. 5 + 500 ul Standard Diluent (1X)
40 ng/ml	Standard No.3	500 ul of Standard No. 4 + 500 ul Standard Diluent (1X)
20 ng/ml	Standard No.2	500 ul of Standard No. 3 + 500 ul Standard Diluent (1X)
10 ng/ml	Standard No. 1	500 ul of Standard No. 2 + 500 ul Standard Diluent (1X)
0 ng/ml	Standard No. 0	Only Standard Diluent (1X)

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. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Octreotide.
3. It is recommended that all Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to low / incorrect results.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. It is advisable to 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. Pipette **100 ul** of **Standards** or **Samples** into the respective wells.

3. Pipette **100 ul** of **Octreotide:HRP Conjugate** into each well.

4. Cover the plate and incubate for 90 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 microplate for 30 minutes at RT 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**. 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 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 Octreotide 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 Octreotide Concentration. If samples were diluted, multiply by the appropriate dilution factor.

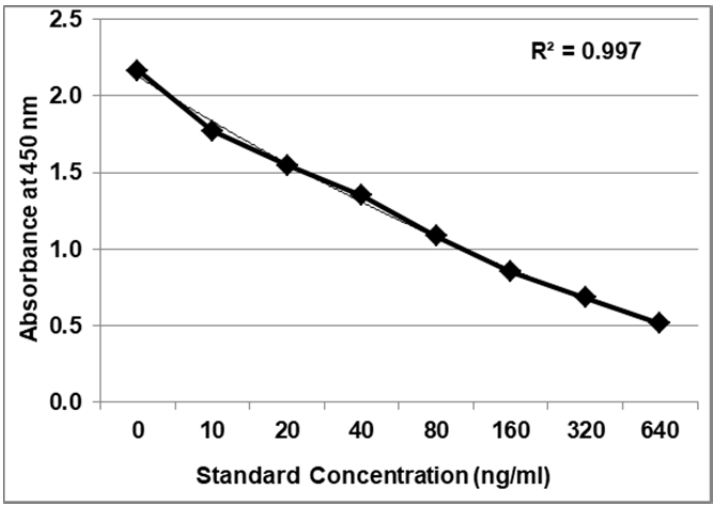
Software which is able to generate a linear regression is best recommended for automated results.

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.
- If the absorbance value is equivalent or higher than the 640 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	2.203	2.122	2.163	0.0	--
10	1.779	1.766	1.773	9.5	94.6
20	1.523	1.574	1.549	21.4	106.8
40	1.326	1.375	1.351	38.6	96.5
80	1.073	1.099	1.086	81.2	101.5
160	0.838	0.870	0.854	162.8	101.7
320	0.652	0.712	0.682	301.4	94.2
640	0.517	0.515	0.516	670.5	104.8

Typical Graph



Abs = absorbance at 450 nm

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 as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

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 10 ng/ml

Specificity:
The antibodies used in the kit are Octreotide antibody with cross-reactivity to Octreotide.

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Octreotide present in matrix. The standard graph range indicated is 0 ng/ml to 640 ng/ml.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (10 ng/ml), medium (160 ng/ml) and high (640 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<12%	<10%
Medium	<8%	<5%
High	<8%	<5%

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.

SCHEMATIC ASSAY PROCEDURE

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

Components (2-8ºC) —30 mins RT Before Use→ 

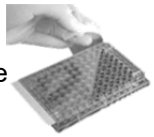
Thaw at Room Temperature (18-24ºC) → USE NOW

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

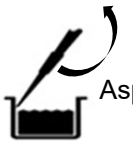
Kit Components RESULTS → AVOID → THAW (22±4ºC) → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → NO CHANGE IN

3.  Pipette **100 ul Standards / Samples** into each well.

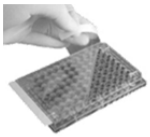
4.  Pipette **100 ul Octreotide HRP Conjugate** into the respective wells.

5. Cover plate  and incubate for 

at 37°C.

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

7.  Pipette **100 ul TMB Substrate** into each well.

8. Cover plate  and incubate for 

at Room Temperature.

9.  Pipette **100 ul Stop Solution** into each well.

10. 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	ug/ml Semaglutide equivalent
1A 2A	0 ng/ml 0 ng/ml			
1B 2B	10 ng/ml 10 ng/ml			
1C 2C	20 ng/ml 20 ng/ml			
1D 2D	40 ng/ml 40 ng/ml			
1E 2E	80 ng/ml 80 ng/ml			
1F 2F	160 ng/ml 160 ng/ml			
1G 2G	320 ng/ml 320 ng/ml			
1H 2H	640 ng/ml 640 ng/ml			
1I 2I	Sample			
1A 2A	Sample			
1B 2B	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 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.

THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Anti-Octreotide Coated Microtiter Plate (12X8 wells)
STD	Octreotide Standard, lyophilized
HRP CONJ	Octreotide HRP conjugate
1X STD DIL	(1X) Standard Diluent
1X SAMP DIL	(1X) Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature