Catalog Number: CUBEK06972

Size: 12 X 8-well Strips (96T)

This ELISA kit applies to the in vitro quantitative determination of Rat δSIP concentrations in serum, plasma and other biological fluids.

Detection Range:	Sensitivity:	Sample Types:
0.156 - 10 ng/mL	0.094 ng/ml	Serum, Plasma, Biological Fluids

Specificity: This kit recognizes natural and recombinant rat rat δSIP. No significant cross-reactivity or interference between rat rat δSIP and analogues was observed.

Storage: Store at 4˚C for 1 month, at -20˚C for up to longer storage. Avoid multiple freeze/thaw cycles (Shipped with blue ice.)

Principle

The Rat δSIP (Delta Sleep Inducing Peptide) ELISA Kit uses the Sandwich-ELISA method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to rat rat δSIP. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the anti-rat rat δSIP antibody. A biotinylated detection antibody specific for rat rat δSIP and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each well, successively, and the plate is incubated at 37˚C. The wells are washed and the substrate solution is added. Wells containing rat rat δSIP biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme substrate is terminated by the addition of a sulfuric acid solution, turning the solution yellow. The optical density (OD) is measured spectrophotometrically at wavelength of 450 nm. The OD value is proportional to the concentration of the rat rat δSIP. You can calculate the concentration of rat rat δSIP in the samples by comparing the OD of the samples to the standard curve.

Notice Before Application

Please read the following instructions before starting the experiment.

1. In order to familiarize yourself with the protocol and determine appropriate sample dilutions, an initial pilot experiment using a few samples is recommended.
2. Prior to using the Kit, spin tubes briefly to ensure no liquid remains in the caps.
3. Duplicates are recommended for both standard and sample testing.
4. Do not let the 96-well plate dry out. If this occurs, the components will become inactive.
5. To avoid cross contamination, do not re-use tips.
6. Use only reagents from the same batch.
7. Pre-warm wash and buffer solutions to 37°C for 30 min prior to use. Using pre-warmed solutions will help reduce any marginal effects due to temperature differences across the plate.

Kit Components

Catalog number	Quantity	Storage
1 x 96-well micro ELISA Plate pre-coated with anti-rat rat δSIP antibody	8 wells ×12 strips	4ºC/-20 ºC
Rat Rat δSIP Reference Standard	2 Tubes	4ºC/-20ºC
Reference Standard & Sample Dilution Buffer	20 ml	4ºC
Biotinylated anti-rat rat δSIP antibody	120 µl	4ºC/-20ºC
HRP Conjugate	120 µl	4ºC (dark)
Biotinylated anti-rat rat δSIP antibody dilution buffer	10 ml	4ºC
Was Buffer (25X)	30 ml	4ºC
Substrate Reagent	10 ml	4ºC
Stop Solution	10 ml	4ºC
Adhesive film	5	RT

Material Required But Not Provided

1. Microplate reader in standard size.
2. An automated plate washer is recommended, but not required.
3. Adjustable pipettes and pipette tips. If working with a large number of samples, we recommend using a multi-channel pipette.
4. Clean tubes and Eppendorf tubes.
5. Absorbent paper.

Protocol Summary

1. Add 100 µl standard or sample to each well. Incubate 90 mintues at 37℃.
2. Add 100 µl Biotinylated anti-rat rat δSIP antibody, Incubate 1 hour at 37℃.
3. Aspirate and wash 3 times.
4. Add 100 µl HRP Conjugate. Incubate 30 minutes at 37℃.
5. Aspirate and wash 5 times.
6. Add 90 µl Substrate Reagent. Incubate 15 minutes at 37℃.
7. Add 50 µl Stop Solution. Read it at 450 nm.
8. Calculate results.

Preparation

1. Sample Preparation and Storage

Store samples to be assayed no more than 48 hours at 2 - 8°C. For long-term storage, aliquot and freeze samples at -20°C (< 1 month) or -80ºC (< 6 months). Avoid repeated freeze/thaw cycles.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifuging for 15 minutes at 1000 × g. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin-free. Assay immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using EDTA or heparin as an anti-coagulant. Centrifuge samples for 15 minutes at 1000 × g at 2 – 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Hemolysis samples are not suitable for ELISA assay.

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant. Assay immediately or aliquot and store samples at -20°C.

Tissue homogenates:

> **Note:** Prior to using Kit, determine the most effective homogenization method for your sample by researching the literature and experimenting on excess samples.

For general information, hemolysis blood may affect results. To remove excess blood thoroughly, mince tissues into small pieces and rinse in ice-cold PBS (0.01 M, pH 7.4). Weigh and then homogenize samples in PBS with a glass homogenizer. Keep samples on ice while homogenizing. Further cell disruption may be accomplished by sonication or subjecting samples to a few, quick freeze/thaw cycles. Centrifuge homogenates for 5 minutes at 5000×g to yield a cleared supernatant.

Other biological fluids: Centrifuge samples for 20 minutes at 1000 × g at 2 – 8°C. Collect the supernatant and carry out the assay immediately.

2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the standard curve. Dilute the sample using the provided **Reference Standard and Sample Dilution Buffer**. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the dilution buffer.**

High target protein concentration (10X Upper Detection Range - >100X Upper Detection Range). The working dilution is 1:100. I.e. Add 1 µl sample into 99 µl sample dilution buffer.

Medium target protein concentration (2X Upper Detection Range - 10X Upper Detection Range). The working dilution is 1:10. I.e. Add 10 µl sample into 90 µl sample dilution buffer.

Low target protein concentration (2X Lower Detection Range - 2X Upper Detection Range). The working dilution is 1:2. I.e. Add 50 µl sample to 50 µl sample dilution buffer.

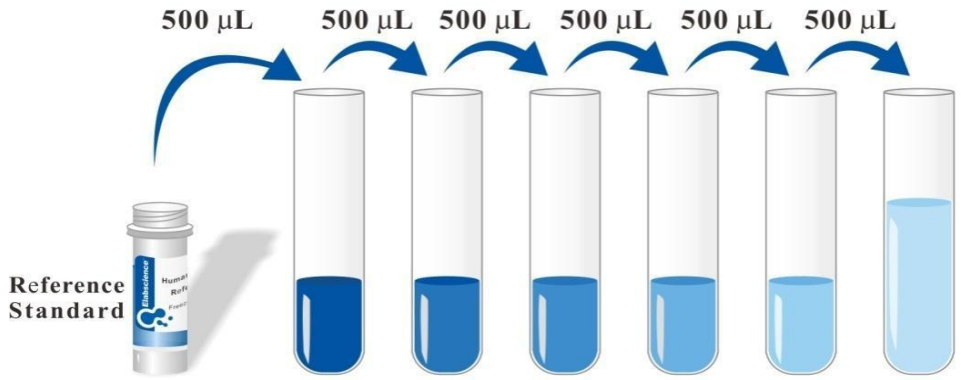
Very Low target protein concentration (0 – 2X Lower Detection Range). No dilution necessary, or the working dilution is 1:2.

3. Reagent Preparation and Storage

A. Reconstitute Rat δSIP standard solution no more than 2 hours prior to the experiment.

Prepare standards at the following concentrations: 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, .63 ng/mL, .31 ng/mL, .16 ng/mL.

1. Label 6 tubes from: 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, .63 ng/mL, .31 ng/mL, .16 ng/mL.
2. Create a 10 ng/mL standard stock solution by adding 1 ml of **Reference Standard and Sample Dilution Buffer** to one tube of standard. Let sit for 10 min at room temp and mix thoroughly. Use one tube of standard per experiment.



Tube	Standard Stock	1	2	3	4	5	6	
ng/mL	10	5	2.5	1.25	0.625	0.3125	0.15625	

3. It is recommended that most standards be created by performing 1:2 serial dilutions. However, an initial, non-1:2 dilution may be necessary. For example: 1) If the standard stock solution concentration is 10,000 pg/ml (10 ng/ml) and the highest required standard is 500 pg/ml, create the 500 pg/ml standard by adding 50 µl of standard stock solution into 950 µl of sample dilution buffer (mix thoroughly). 2) If the standard concentration is 20,000 pg/ml and the highest required standard is 8000 pg/ml, create the 8000 pg/ml standard by adding 400 µl of standard stock solution into 600 µl of sample dilution buffer (mix thoroughly).
4. Perform all other standard dilutions by aliquoting 0.5 ml of standard into 0.5 ml of sample dilution buffer, mixing each standard thoroughly, as it is prepared.

Note: The standard solutions are best used within 2 hours. The standard stock solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-Rat Rat δSIP antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1 - 0.2 ml more than total volume)
2. Biotinylated anti-Rat Rat δSIP antibody should be diluted in 1:100 with the antibody dilution buffer and mixed thoroughly. (i.e. Add 1 µl Biotinylated anti-Rat Rat δSIP antibody to 99 µl antibody dilution buffer.)

C. Preparation of HRP Conjugate: The solution should be prepared no more than 1 hour prior to the experiment.

1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1 - 0.2 ml more than total volume)
2. HRP Conjugate should be diluted in 1:100 with the HRP Conjugate dilution buffer and mixed thoroughly. (i.e. Add 1 µl HRP Conjugate to 99 µl HRP Conjugate dilution buffer.)

Assay Procedure

When diluting samples and reagents, mix thoroughly to ensure a homogenous solution. A standard detection curve should be prepared for each experiment. The user will decide sample dilutions.

1. Aliquot 0.1 ml per well of the 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, .63 ng/mL, .31 ng/mL, .16

ng/mL solutions into the pre-coated 96-well plate. Add 0.1 ml of the sample dilution buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample to each empty well. **See "Sample Dilution Guideline" above for details.** It is recommended that each standard solution and each sample be measured in duplicate.

2. Seal the plate with a new adhesive cover provided and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-rat rat δSIP antibody working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 60 min.
5. Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350 µl) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against clean absorbent paper.
6. Add 0.1 ml of HRP Conjugate working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 30 min.
7. Wash plate 5 times with Wash Buffer. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of Substrate Solution into each well, seal the plate with a new adhesive cover and incubate at 37°C in dark for 15 min (**Note:** For reference only, the optimal incubation time can be shorter than 15 min and as long, but no longer than 30 min. When an apparent gradient appears in the standard wells, the reaction should be terminated.
9. Add 50 µl of Stop Solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat rat δSIP concentration of the samples can be interpolated from the standard curve. **Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from the interpolation to obtain the concentration before dilution.

Note: *This kit is designated for **research use only** and not to be used for clinical diagnosis.*