Technical Manual

Rat Sst (Somatostatin) ELISA Kit

- Catalogue Code: RTFI00214
- Sandwich ELISA Kit
- Research Use Only

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1. Key features and Sample Types

Aliases:

Sst, SMST, SOM, SRIF, Growth hormone release-inhibiting factor, somatostatin-14, somatostatin-28

Uniprot:

P60042

Detection method:

Sandwich, Double Antibody

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Rat

Range:

15.625-1000pg/ml

Sensitivity:

< 9.375pg/ml

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

The Assay Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands. Our assay kits use a quantitative sandwich ELISA technique and each kit comes with highly specific antibodies pre-coated onto a 96-well microtiter plate.

At Assay Genie we understand the need for speed! Therefore, we have developed an ultrafast protocol for rapid results. Once you have prepared and plated your samples, blanks and standards, you simply incubate with the specific biotin-conjugated primary antibody and Avidin conjugated Horseradish Peroxidase (HRP). After plate washing and addition of the TMB (3,3',5,5'-Tetramethylbenzidine) solution, the appearance of a blue colour is detected due to an enzymatic reaction catalysed by HRP. Next the addition of the Stop Solution terminates the HRP reaction and the blue colour turns yellow with the signal intensity measured on a plate reader at 450nm. The amount of bound analyte is proportional to the signal generated by the reaction meaning the kit assay gives you a quantitative measurement of the analyte in your samples.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	2-8°C/-20°C
2	Lyophilized Standard	1 vial	2 vials	2-8°C/-20°C
3	Sample Dilution Buffer	10 mL	20 mL	2-8°C
4	Biotin-labelled Antibody (Lyophilized)	1 vial	1 vial	2-8°C (Avoid Direct Light)
5	Purified Water		200 uL	2-8°C
6	Antibody Dilution Buffer	5 mL	10 mL	2-8°C
7	HRP-Streptavidin Conjugate (SABC)	60 uL	120 uL	2-8°C (Avoid Direct Light)
8	SABC Dilution Buffer	5 mL	10 mL	2-8°C
9	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
10	Stop Solution	5ml	10ml	2-8°C
11	Wash Buffer (25x)	15 mL	30 mL	2-8°C
12	Plate Sealer	3 pieces	5 pieces	
13	Manual	1	1	

Additional materials required:

1. 37°C incubator
2. Plate Reader with 450nm filter
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

5. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolyzed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1000 x g. Collect the clear supernatant and assay immediately.

Cell lysates: Commercial RIPA kits are recommended for preparation of cell lysate samples. For 2 x 10^6 cells, lyse cells in 0.5 mL RIPA buffer, removing DNA content. Determine the protein concentration using a BCA assay kit (BN01031) with each sample concentration being ≤ 300μg for analysis.

Tissue Homogenates: Rinse tissue with 1X PBS to remove excess blood. Mince tissue after weighing it and homogenize in PBS (the volume depends on the weight of the tissue. 9mL PBS (including protease inhibitors) would be appropriate for 1 gram of tissue. To further lyse cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 x g and the supernatant is removed for assaying. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolysed samples are not suitable for this assay.

6. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350μl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350μl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: **Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.**

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

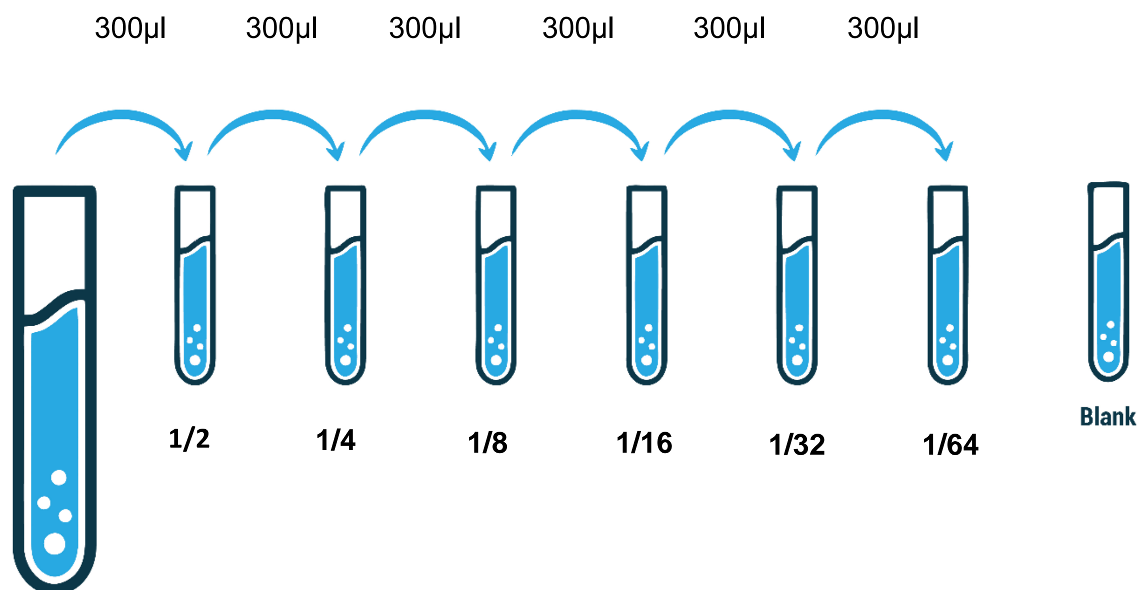
Dilute 30 ml (15 ml for 48T) of Concentrated Wash Buffer into 750 ml (375 ml for 48T) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

2. Standard Dilution:

1). Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 10 min and mix thoroughly.

2). Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 300μl of the Standard dilution buffer into each tube. Add 300μl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 300μl from 1st tube to 2nd tube and mix thoroughly. Transfer 300μl from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample dilution buffer is used as blank control.

DILUTION SERIES



Note: The standard solutions are best used within 2 hours. The standard solution series should be kept at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of biotin-labelled antibody working solution:

Prepare within 1 hour before the experiment.

1. Dissolve: Add 70ul purified water into tube and mix them thoroughly, after the biotin-labelled antibody is dissolved, please store it at 2-8°C.
2. Calculate the total volume of the working solution: 50μl / well × quantity of wells (Allow 100-200μl more than the total volume).
3. Dilute the biotin-detection antibody with antibody dilution buffer at 1:100 and mix thoroughly (i.e. Add 1μl of biotin-labelled antibody into 99μl of antibody dilution buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30 minutes of starting the experiment.

1. Calculate the total volume of the working solution: 100μl / well × quantity of wells. (Allow 100-200μl more than the total volume)
2. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1μl of SABC into 99μl of SABC dilution buffer.)

7. Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (blank) wells!
2. Add Sample and Biotin-**labelled** Antibody: Add 50ul of Standard, Blank, or Sample per well. The blank well is added with Sample/Standard Dilution Buffer. Immediately add 50ul Biotin-**labelled** Antibody Working Solution into each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)
3. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
4. HRP-Streptavidin Conjugate (SABC): Add 100ul SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.
5. Wash: Remove the cover and wash plate 5 times with Wash **Buffer and** let the wash buffer stay in the wells for 1-2 minutes each time.
6. TMB Substrate: Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C.). (Note: The reaction time can be shortened or extended according to

the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)

7. Stop: Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation, the standard curve can be plotted as the O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

8. Data Analysis

Calculate using the following equation:

The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of blank well)

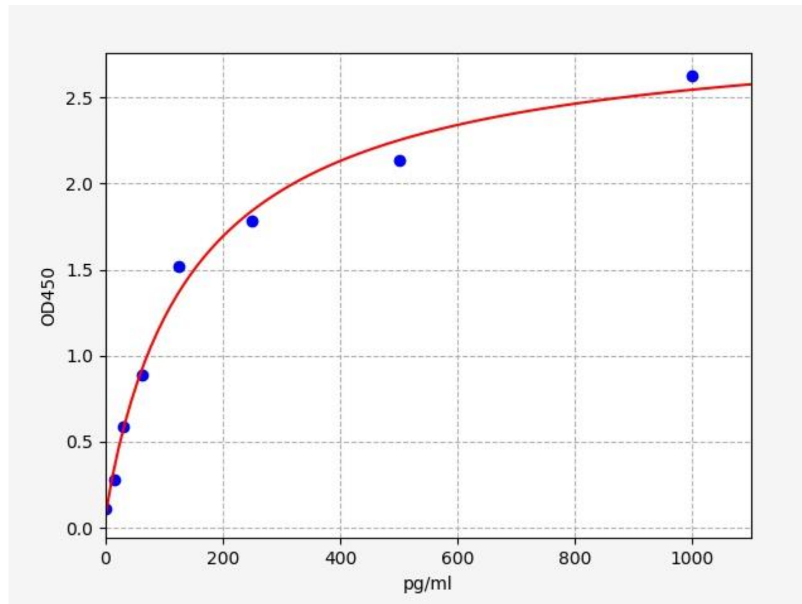
The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as curve expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

9. Typical Data & Standard Curve

Standard Curve

Results of a typical standard run of a ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



Specificity

This assay has high sensitivity and excellent specificity for detection of Rat Sst. No significant cross-reactivity or interference between Rat Sst and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Rat Sst and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Rat Sst and the recovery rates were calculated by comparing the measured value to the expected amount of Rat Sst in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	88-102	95
EDTA Plasma(n=5)	86-104	94
Heparin Plasma(n=5)	88-102	97

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Rat Sst and their serial dilutions.

Sample	1:2	1:4	1:8
Serum(n=5)	86-102%	88-104%	85-103%
EDTA Plasma(n=5)	84-93%	89-98%	83-99%
Heparin Plasma(n=5)	80-91%	81-95%	91-100%

Precision

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

Stability

The stability of the Rat Sst ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

Standard (n=5)	**37°C for 1 month**	**4°C for 6 months**
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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