Mouse Somatostatin (SS) ELISA Kit

EJ1515509

Storage 2-8℃.
Shipping Low temperature transportation.

Introduction

Somatostatin, also known as growth hormone-inhibiting hormone (GHIH), is a polypeptide hormone. It regulates the endocrine system, affects neurotransmission and cell proliferation by interacting with G protein-coupled somatostatin receptors and inhibiting the release of numerous secondary hormones. Somatostatin inhibits the secretion of insulin and glucagon. There are two active forms of somatostatin, produced by alternative cleavage of a prepro-protein: one consisting of 14 amino acids and the other consisting of 28 amino acids. In vertebrates, there are six distinct somatostatin genes, designated as SS1, SS2, SS3, SS4, SS5 and SS6. These six different genes, together with five distinct somatostatin receptors, confer a wide functional range to somatostatin.

Experimental Principle

This kit adopts a competitive enzyme-linked immunosorbent assay (ELISA). Samples, standards, biotin-labeled antibody and HRP conjugate are sequentially added to the microwells pre-coated with mouse somatostatin (SS) antigen. After incubation and washing, the substrate TMB is used for color development. TMB is converted to blue under the catalysis of horseradish peroxidase (HRP), and then converted to the final yellow color under the action of acid. The color intensity is inversely correlated with the level of mouse somatostatin (SS) in the sample. The absorbance (OD value) is measured at 450 nm wavelength using a microplate reader, and the sample concentration is calculated accordingly.

Precautions

1. Strictly follow the specified time and temperature for incubation to ensure accurate results. All reagents must be brought to room temperature (20-25 ℃) before use. Store reagents in the refrigerator immediately after use.
2. Improper washing may lead to inaccurate results. Ensure wells are thoroughly drained before adding substrate. Avoid allowing microwells to dry for extended periods during the procedure.
3. Clean residual liquid and fingerprints from the well bottoms, as these can affect OD values.
4. Substrate developing solution should be colorless; blue-tinted substrate must not be used.
5. Avoid cross-contamination between reagents and samples to prevent erroneous results.
6. Protect from direct strong light during storage and incubation.
7. Do not allow any reaction reagents to come into contact with bleaching agents or their

vapors. Any bleaching compounds will inactivate the biological activity of the kit reagents.

8. Do not use expired products. Components from different catalog or batch numbers must not be mixed.
9. Recombinant proteins from sources other than this kit may not be recognized by the kit antibodies.
10. All samples should be handled with care and processed and tested in accordance with established protocols if there is a risk of disease transmission.

Kit Composition

EJ1515509	Component	48T	96T	Storage
EJ1515509A	Pre-coated Assay Plate	48 wells	96 wells	2-8℃.
EJ1515509B	Standard	1 vial	2 vials	2-8℃.
EJ1515509C	Universal Diluent	1×20 mL	2×20 mL	2-8℃.
EJ1515509D	Biotin-antibody (100×)	30 µL	60 µL	2-8℃.
EJ1515509E	Streptavidin-HRP (100×)	60µL	120 µL	2-8℃.
EJ1515509F	Wash Buffer (20×)	1×10 mL	2×10 mL	2-8℃.
EJ1515509G	TMB Substrate	5 mL	10 mL	2-8℃.
EJ1515509H	Stop Solution	3 mL	6 mL	2-8℃.
EJ1515509I	Plate Sealer	4 sheets	4 sheets	2-8℃

Sample Handling and Requirements

1. The kit's detection range does not equate to the concentration range of the analyte in the sample. It is recommended to estimate the analyte concentration in the sample using relevant literature and confirm the actual concentration via a preliminary experiment. If the analyte concentration is too high or too low, dilute or concentrate the sample appropriately.
2. If the sample type is not listed in the manual, a preliminary experiment is recommended to verify detection validity.
3. Serum: Allow whole blood collected in serum separation tubes to stand at room temperature for 2 hours or overnight at 2-8 ℃, then centrifuge at 1000 × g for 20 minutes and collect the supernatant. Alternatively, store the supernatant at −20 ℃ or −80 ℃, avoiding repeated freeze-thaw cycles.
4. Plasma: Collect samples using EDTA or heparin as anticoagulant, centrifuge at 1000 × g for 15 minutes at 2-8℃ within 30minutes of collection, and use the supernatant for testing. Alternatively, store the supernatant at −20℃ or −80℃, avoiding repeated freeze-thaw cycles.
5. Tissue homogenate: Rinse tissue with pre-chilled PBS (0.01 M, pH=7.4) to remove

residual blood (lysed red blood cells in homogenate will interfere with results). Weigh and mince the tissue. Place minced tissue and an appropriate volume of PBS (typically 1:9 w/v, e.g., 1 g tissue + 9 mL PBS; volume can be adjusted as needed and recorded) into a glass homogenizer, and grind on ice or using a homogenizer. For further cell lysis, sonicate or subject the homogenate to repeated freeze-thaw cycles. Centrifuge the homogenate at 5000 × g for 5-10 minutes and use the supernatant for testing.

6. Cell culture supernatant: Centrifuge at 1000 × g for 20 minutes and use the supernatant for testing. Alternatively, store the supernatant at −20 ℃ or −80 ℃, avoiding repeated freeze-thaw cycles.
7. Cell lysate: Gently wash adherent cells with pre-chilled PBS, then digest with trypsin and collect cells by centrifugation at 1000 × g for 5 minutes. Suspension cells can be collected directly by centrifugation. Wash collected cells three times with pre-chilled PBS, resuspend in 150-200 µL PBS per 1× 10^6 cells (add protease inhibitors to PBS if recommended; reduce PBS volume if analyte is low), and lyse cells by repeated freeze-thaw or sonication. Centrifuge the extract at 1500 × g for 10 minutes at 2-8 ℃ and use the supernatant for testing.
8. Other biological samples: Centrifuge at 1000 × g for 20 minutes and use the supernatant for testing.
9. Sample appearance: Samples should be clear and transparent; remove any suspended matter by centrifugation.
10. Sample storage: If testing within 1 week, store samples at 4 ℃. If testing is delayed, aliquot and store at −20 ℃ (for testing within 1 month) or −80 ℃ (for testing within 6 months), avoiding repeated freeze-thaw cycles. Hemolyzed samples should not be used as hemolysis interferes with results.

Sample Dilution Protocol

Estimate the sample concentration range in advance. If sample dilution is required, follow the protocol below:

1. 100-fold dilution: One-step dilution. Add 5 µL sample to 495 µL general diluent.
2. 1000-fold dilution: Two-step dilution. Add 5 µL sample to 95 µL general diluent (20-fold), then add 5 µL of the 20-fold dilution to 245 µL general diluent (50-fold); total dilution=1000-fold.
3. 100,000-fold dilution: Three-step dilution. Add 5 µL sample to 195 µL general diluent (40-fold), then add 5 µL of the 40-fold dilution to 245 µL general diluent (50-fold), then add 5 µL of the 2000-fold dilution to 245 µL general diluent (50-fold); total dilution = 100,000-fold.

In each step, use at least 3 µL for pipetting and do not exceed 100-fold dilution per step. Mix thoroughly after each dilution and avoid bubbles.

Required Self-Provided Equipment

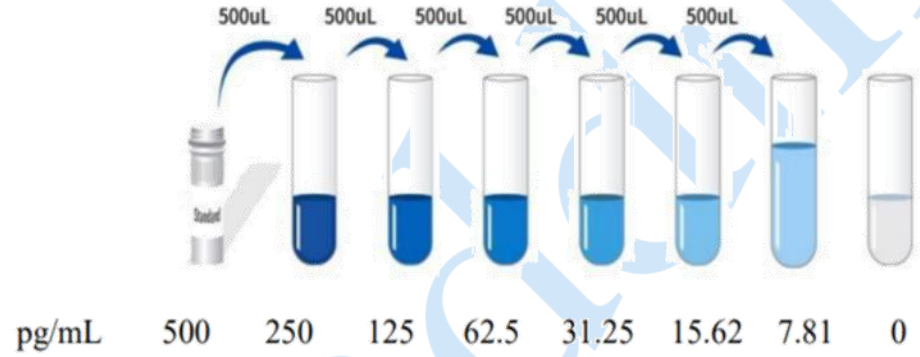
1. Microplate reader (450 nm).

2. High-precision pipettes and tips: 0.5-10 μL, 5-50 μL, 20-200 μL, 200-1000 μL.
3. 37℃ incubator.
4. Distilled or deionized water.

Pre-Test Preparation

1. Remove the kit from the refrigerator 10 minutes in advance and equilibrate it to room temperature.
2. Preparation of standard serial working solutions: Add 1 mL of universal diluent to the lyophilized standard, allow it to stand for 15 minutes until fully dissolved, then mix gently (concentration: 500 pg/mL). Subsequently, perform serial dilutions to the following concentrations: 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.62 pg/mL, 7.81 pg/mL, and 0 pg/mL.

Serial dilution method: Take 7 EP tubes, add 500 μL of universal diluent to each tube. Pipette 500 μL from the 500 pg/mL standard working solution into the first EP tube, mix well to prepare 250 pg/mL standard working solution. Repeat this pipetting and mixing step sequentially for subsequent tubes. The last tube is directly used as the blank well, and no liquid needs to be transferred from the penultimate tube, as shown in the figure below.



3. Preparation of Biotin-Antibody Working Solution: Centrifuge the 100× concentrated Biotin-Antibody at 1000×g for 1 minute 15 minutes before use. Dilute the 100× concentrated Biotin-Antibody to 1× working concentration with universal diluent (e.g., 10 μL concentrated solution + 990 μL universal diluent). Prepare freshly before use.
4. Preparation of enzyme conjugate working solution: Centrifuge the 100× concentrated enzyme conjugate at 1000 × g for 1 minute 15 minutes before use. Dilute the 100× concentrate to 1× working concentration with general diluent (e.g., 10 μL concentrate + 990 μL diluent). Prepare fresh.
5. Preparation of 1× washing solution: Add 10 mL of 20× washing solution to 190 mL distilled water (Crystals may form in the concentrated washing solution stored in the refrigerator; this is normal. Allow to reach room temperature and dissolve completely before preparation).

Procedure

1. Remove required strips from the aluminum foil bag that has been equilibrated at room temperature for 10 minutes. Seal the remaining strips in a self-sealing bag and return to 4℃.
2. Sample addition: Add 50 μL of samples or standards of different concentrations to corresponding wells respectively. Add 50 μL of universal diluent to the blank well. Then add 50 μL of Biotin-antibody working solution to each well. Cover with sealing film and incubate at 37 ℃ for 60 minutes. (Suggestion: Dilute test samples at least 1-fold with universal diluent before adding to the microplate to reduce matrix effect on results. Multiply by the corresponding dilution factor when calculating final concentration. Duplicate wells are recommended for all test samples and standards.)
3. Washing: Discard the liquid, add 300 μL of 1× washing solution to each well, let stand for 1 minute, discard the washing solution, and pat dry on absorbent paper. Repeat washing 3 times (or use an automatic plate washer).
4. Addition of enzyme conjugate working solution: Add 100 μL of enzyme conjugate working solution to each well. Cover with sealing film and incubate at 37 ℃ for 30 minutes.
5. Washing: Discard the liquid and wash 5 times according to the method in step 3.
6. Substrate addition: Add 90 μL of substrate (TMB) to each well, cover with sealing film, and incubate at 37 ℃ in the dark for 15 minutes.
7. Stop solution addition: Take out the microplate, add 50 μL of stop solution directly to each well, and measure the OD value of each well at 450 nm immediately.

Calculation of Experimental Results

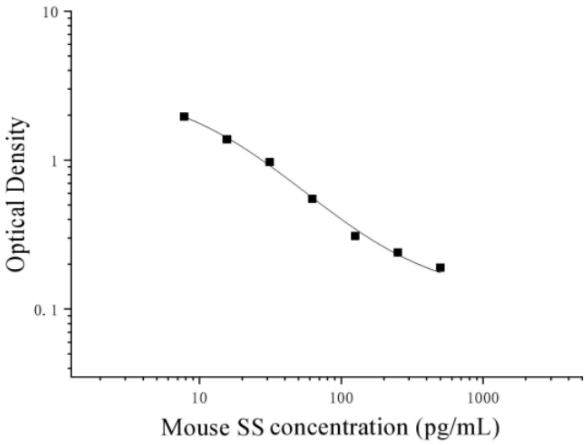
Result Judgment:

1. Calculate the average OD value of duplicate wells for standards and samples. Using concentration as the abscissa and OD value as the ordinate, draw a standard curve of four-parameter logistic function on a log-log coordinate paper (exclude the blank group value during plotting).
2. If the sample OD value is higher than the upper limit of the standard curve, the sample should be properly diluted and re-measured, and the corresponding dilution factor shall be multiplied when calculating the sample concentration.

Typical Data and Reference Curve

The following data and curve are for reference only. The experimenter should establish a standard curve based on their own experiment.

Concentration (pg/mL)	500	250	125	62.5	31.25	15.62	7.81	0
OD Value	0.19	0.24	0.31	0.55	0.97	1.38	1.96	2.52



Note: This figure is for reference only. The sample content should be calculated using the standard curve plotted from the experimental data of each run.

Kit Performance

1. Repeatability: Intra-assay CV< 10%; inter-assay CV< 10%.
2. Recovery rate: Mouse SS at three different concentration levels was spiked into selected healthy mouse serum, plasma and cell culture supernatant, and the recovery rate was calculated.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	81-101	96
Plasma (n=8)	92-105	101
Cell culture supernatant (n=8)	97-108	103

3. Linear dilution: High-concentration mouse SS was spiked into 4 selected healthy mouse serum, plasma and cell culture supernatant samples respectively. Serial dilutions were performed within the dynamic range of the standard curve to evaluate linearity.

Dilution	Ratio Recovery (%)	Serum	Plasma	Cell culture supernatant
1: 2	Range	83-95	88-96	90-111
1: 2	Average recovery	91	93	97
1: 4	Range	89-104	87-108	105-114
1: 4	Average recovery	93	98	109

Troubleshooting

If experimental results are unsatisfactory, photograph the color development, save experimental data, retain the used strips and unused reagents, and contact our technical support for assistance. You may also refer to the following:

Problem Description	Possible Cause	Recommended Solution
Poor standard curve linearity	Incorrect standard dilution	Ensure standards are dissolved and diluted as recommended
Poor standard curve linearity	Inaccurate pipetting	Calibrate pipettes regularly and check tip sealing
Poor standard curve linearity	Evaporation of reaction solution	Seal the plate with a film
Poor standard curve linearity	Inadequate washing	Adequate washing times and sufficient washing solution volume
Poor standard curve linearity	Foreign matter on well bottoms	Clean well bottoms before reading
Weak or no color development	Weak or no color development	Insufficient incubation time Ensure full incubation time
Weak or no color development	Incorrect incubation temperature	Incubate at the recommended temperature
Weak or no color development	Insufficient reagent volume	Check pipettes and follow the protocol strictly
Weak or no color development	Incorrect dilution	Verify reagent dilution steps
Weak or no color development	Inactivated enzyme conjugate	Mix conjugate and substrate and check for color development
Low OD values	Incorrect microplate reader settings	Check instrument wavelength
Low OD values	No stop solution added	Add appropriate amount of stop solution
Low OD values	Too long a wait before reading	Read the plate promptly
Low OD values	Sample concentration too high	Determine appropriate dilution via preliminary experiment
Low OD values	Sample concentration too low	Determine appropriate dilution via preliminary experiment
High background	Contaminated Substrate solution	Replace Substrate solution
High background	Excessive color development time	Control color development time
High background	Incorrect dilution of detection antibody or enzyme conjugate	Use recommended dilution method
High background	Inadequate washing	Adequate washing times and sufficient washing solution volu