

## Human Somatostatin ELISA Kit

### Cat#: orb1214370 (ELISA Manual)

Cat.No orb1214370

Standard Curve Range 1-400ng/L

Sensitivity 0.53ng/L

Size 48T, 96T

Storage Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

\*This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.

#### Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra assay precision. We measured random samples of E1062Hu within the same batch/lot to ensure the consistency of the kits' performances.

Intra-assay	Sample	n	Mean	Standard deviation	CV (%)
Intra-Assay	1	18	82.6	3.72	4.5
Intra-Assay	2	18	54.2	3.68	6.8
Intra-Assay	3	18	17.7	1.25	7.1

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

$CV(\%) = SD/mean \times 100$

Inter-Assay:  $CV < 10\%$

#### Intended Use

This Sandwich kit is for the accurate quantitative detection of Human Somatostatin (also known as SST) in serum, plasma, cell culture supernatants, Ascites, tissue homogenates or other biological fluids.

#### Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human SST antibody. SST present in the sample is added and binds to antibodies coated on the wells. And then

biotinylated Human SST Antibody is added and binds to SST in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated SST antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human SST. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

### Reagent Provided

Components	Quantity (96T)	Quantity (48T)
Standard solution (480ng/L)	0.5ml x1	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard diluent	3ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Stop solution	6ml x1	3ml x1
Substrate solution A	6ml x1	3ml x1
Substrate solution B	6ml x1	3ml x1
Wash buffer Concentrate (25x)	20ml x1	20ml x1
Biotinylated Human SST antibody	1ml x1	1ml x1
User instruction	1	1
Plate sealer	2 pics	2 pics

### Material Required but Not Supplied

37°C±0.5°C incubator  
 Absorbent paper  
 Precision pipettes and disposable pipette tips  
 Clean tubes  
 Deionized or distilled water  
 Microplate reader with 450 ± 10nm wavelength filter

### Precautions

Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes. This instruction must be strictly followed in the experiment. Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use. Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.

Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination. Avoid using the reagents from different batches together. Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time. Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent. The kit should not be used beyond the expiration date.

#### Specimen Collection

**Serum** Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

**Plasma** Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.

**Cell culture supernatant** Collect by sterile tubes. When detecting secreted components, centrifuge at 2000-3000 RPM for 20 minutes.

Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components.

Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

**Tissue** Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 15 minutes at 12,000 RPM at 4°C to get the supernatant. Avoid freeze/thaw cycles.

**Urine/Ascites/Cerebrospinal fluid** Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

#### Note

Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must contact us to determine the optimal sample for user's particular experiment.

Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 3 months. Avoid repeated freeze thaw cycles.

Samples should be brought to room temperature before starting the assay.

Centrifuge to collect sample before use.

Samples containing NaN<sub>3</sub> can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).

Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

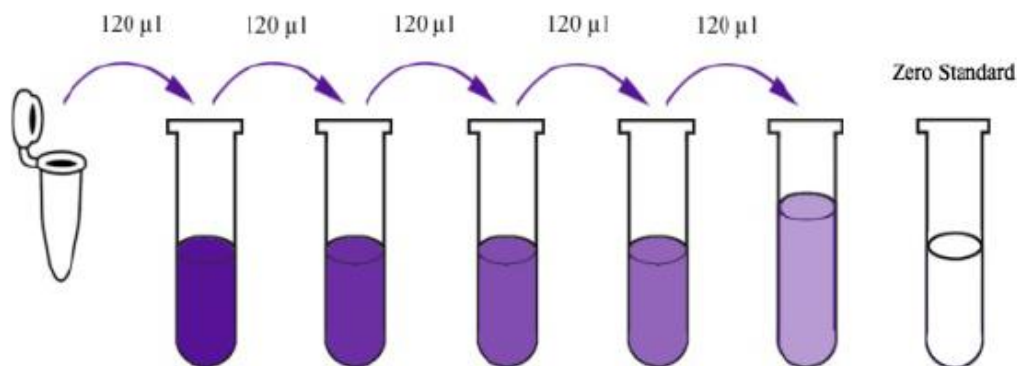
Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

#### Reagent Preparation

All reagents should be brought to room temperature before use.

Standard Reconstitute the 120ul of the standard (480ng/L) with 120ul of standard diluent to generate a 240ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (240ng/L) 1:2 with standard diluent to produce 120ng/L, 60ng/L, 30ng/L and 15ng/L solutions. Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

240ng/L	Standard No.5	120ul Original standard + 120ul Standard diluent
120ng/L	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
60ng/L	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
30ng/L	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
15ng/L	Standard No.1	120ul Standard No.2 + 120ul Standard diluent



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
480ng/L	240ng/L	120ng/L	60ng/L	30ng/L	15ng/L

Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

### Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50ul standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4. Add 40ul sample to sample wells and then add 10ul Human SST antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50ul Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

### Summary

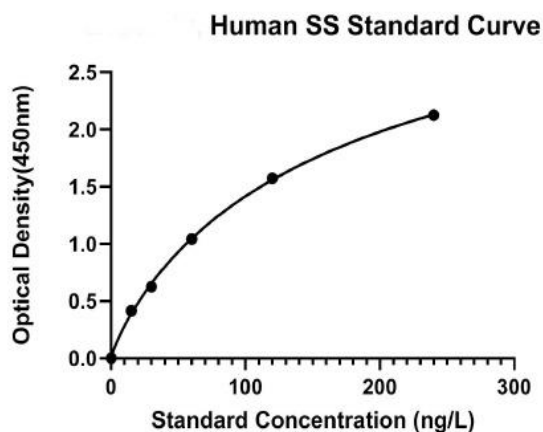
1. Prepare all reagents, samples and standards.
2. Add sample and ELISA reagent into each well. Incubate for 1 hour at 37°C.
3. Wash the plate 5 times.
4. Add substrate solution A and B. Incubate for 10 minutes at 37°C.
5. Add stop solution and color develops.
6. Read the OD value within 10 minutes.

### Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

### Typical Data

The standard curve of orb1214370 is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Concentration	O.D	Average	Corrected
240ng/L	2.035	2.169	2.126
	2.302		
120ng/L	1.632	1.618	1.574
	1.603		
60ng/L	1.084	1.086	1.042
	1.087		
30ng/L	0.648	0.669	0.626
	0.69		
15ng/L	0.442	0.458	0.415
	0.474		
0ng/L	0.047	0.043	0
	0.039		

## Troubleshooting

### High Background possible case

Improper washing  
 Incorrect incubation temperature  
 Incubation time too long  
 Substrate exposed to light prior to use  
 Substrate was contaminated

Contaminated wash buffer

### Solution

Increasing duration of soaking steps  
 Incubate at 37°C  
 Reduce incubation time  
 Keep substrate in a dark place  
 Replace substrate. Substrate should be clean and avoid crossed contamination by using the sealer  
 Use a clean buffers and sterile filter

### Weak or No Signal possible case

A reagent or a step of the procedure omitted by mistake  
 Antibody are not enough  
 Improper washing  
 Reagent are contaminated  
 Pipette are not clean  
 HRP was not added  
 Sample contains sodium azide  
 Wrong incubation time or temperature

### Solution

Check protocol and follow steps carefully  
 Increase the concentration of the antibody  
 Increasing duration of soaking steps  
 Use new one  
 Pipette should be clean  
 Add HRP according to the instruction  
 Don't prepare samples with sodium azide  
 Check and follow protocol. Place plates in an incubator during incubation periods (set to 37°C).

**Poor Precision possible case**

Pipetting error

Incomplete washing

Unclean wells

**Poor standard curve possible case**

Incorrect preparation of standard

Capture Antibody did not bind

Inefficient washing

Pipetting error

Incorrect storage of components

**Solution**

Dispense quickly and identically into the side of each well. Use calibrated pipettes. Make sure wells are washed adequately by filling the wells with proper amount of wash buffer.

Inspect wells and remove debris prior to use. Wipe the outer bottom of plate clean to remove any debris or fingerprints prior to reading.

**Solution**

Reconstitute standard as suggested on data sheet.

Use Biorbyt ELISA plate in the kit

Be sure wash apparatus is working properly (i.e. distributing even volumes into each well). Be sure wells are empty after aspiration, yet be sure to fill wells in a timely manner.

Dispense quickly and identically into the side of each well. Use calibrated pipettes. Store all components as recommended on data sheet. Do not allow reconstituted reagents to stay at room temperature for excess time.