Product Manual

Revision date: 09/Jan/2018

Human Secretin (SCT) ELISA Kit

Catalog No.: abx350985

Size: 96T

Range: 0.156 ng/ml - 10 ng/ml

Sensitivity: 0.094 ng/ml

Storage: Store at 4°C for up to 6 months. For long term storage, the ELISA plate, Standards and Biotin conjugated antibody can be stored at -20°C.

Application: For quantitative detection of SCT in Human Serum, Plasma and other biological fluids.

Introduction: Secretin is a hormone that regulates water homeostasis throughout the body and influences the environment of the duodenum by regulating secretions in the stomach, pancreas, and liver. It is a peptide hormone produced in the S cells of the duodenum, which are located in the intestinal glands. In humans, the secretin peptide is encoded by the SCT gene. Secretin helps regulate the pH of the duodenum by (1) inhibiting the secretion of gastric acid from the parietal cells of the stomach and (2) stimulating the production of bicarbonate from the centroacinar cells and intercalated ducts of the pancreas. It also stimulates bile production by the liver; the bile emulsifies dietary fats in the duodenum so that pancreatic lipase can act upon them. Meanwhile, in concert with secretin's actions, the other main hormone simultaneously issued by the duodenum, cholecystokinin, is stimulating the gallbladder to contract, delivering its stored bile for the same reason.

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to SCT is pre-coated onto a 96-well plate. The standards and test samples are added to the wells and washed with wash buffer. Biotin conjugated antibody specific to SCT is used as a detection antibody. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the SCT amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of SCT can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standards: 6 × 0.5 ml (5, 2.5, 1.25, 0.625, 0.313, 0.156 ng/ml)
3. Detection antibody: 6 ml
4. HRP-conjugated SCT: 6 ml
5. TMB substrate reagent A: 7 ml
6. TMB substrate reagent B: 7 ml
7. Stop solution: 7 ml
8. Wash buffer (20X): 15 ml
9. Plate sealer: 5 pieces

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Distilled or deionized water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

Sample dilution guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (100 ng/ml - 1000 ng/ml), dilute 1:100, for medium concentration (10 ng/ml - 100 ng/ml), dilute 1:10 and for low concentration (0.156 ng/ml - 10 ng/ml), dilute 1:2. Very low concentrations (≤ 0.156 ng/ml) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 25 ml of concentrated wash buffer into 725 ml of distilled water).

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. **Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.**
2. Add 50 µl of the prepared standards solutions into the standard wells.
3. Leave the control (zero) wells without any liquid.
4. Add 50 µl of appropriately diluted sample into test sample wells.
5. Immediately add 50 µl of HRP-conjugated SCT into each well, followed by 50 µl of Detection antibody. Add the solution at the bottom of each well without touching the side wall.
6. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 1 hour.

7. Remove the cover, and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of three times.

Please note: For automated washing, discard the solution in all wells and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

8. Add 50 µl of TMB substrate reagent A and 50 µl of TMB substrate reagent B into each well. Gently tap the plate to mix thoroughly. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
9. Add 50 µl of Stop solution into each well (including the blank well). There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between SCT concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of SCT were tested 20 times on one plate,

respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of SCT were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<10%