



rHuman Sonic Hedgehog/Shh ELISA Kit

Catalog Number: CEA-C239

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures
IMPORTANT: Please carefully read this user guide before performing your experiment.

rHuman Sonic Hedgehog/Shh ELISA Kit User Guide

Product information

This kit is specifically designed for the accurate quantitation of rHuman Sonic Hedgehog, Suitable for the detection of samples in BSS.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA239-C01	Pre-coated Anti-Shh Antibody Microplate	1 plate
CEA239-C02	rHuman Shh Standard	20 ng×2
CEA239-C03	Biotin-Anti-Shh Antibody Con. Solution	100 µL
CEA239-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA239-C05	Streptavidin-HRP Con. Solution	500 µL
CEA239-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA239-C07	20× Washing Buffer	50 mL
CEA239-C08	Sample Dilution Buffer	15 mL×2
CEA239-C09	Substrate Solution	12 mL
CEA239-C10	Stop Solution	6 mL

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date. For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-Shh Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
rHuman Shh Standard	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.

NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

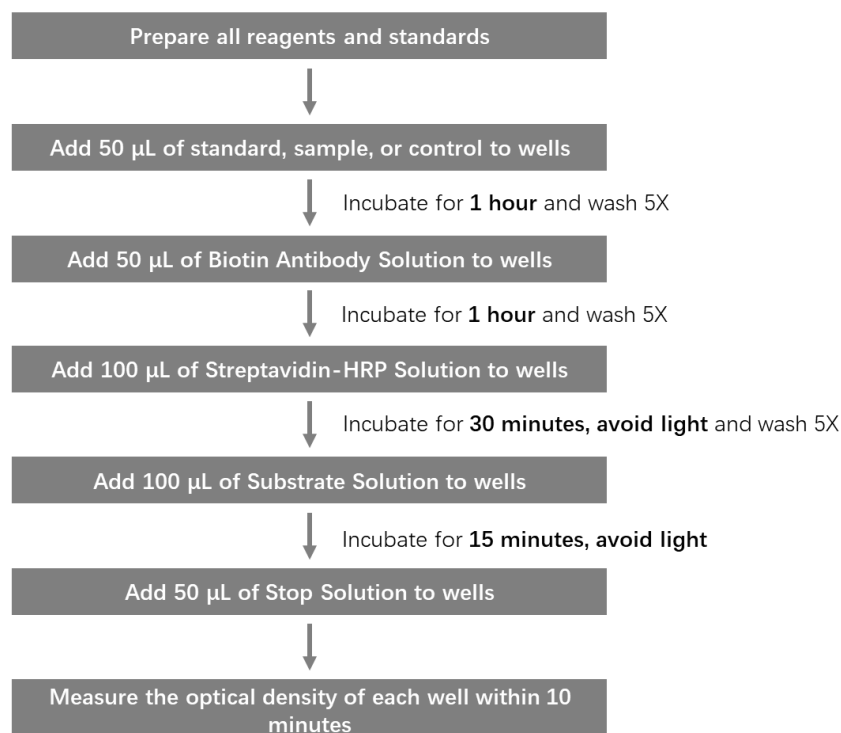
IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in a 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water/Tween 20 (Sinopharm Chemical Reagent Co., Ltd. Chemically pure, Cat No.30189328)
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte: Sonic Hedgehog/Shh



NOTE: Incubation temperature is 18 °C-25 °C

Prepare the working buffers and standard dilutions.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin Antibody Solution: Add 60 µL of Biotin-Anti-Shh Antibody Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Streptavidin-HRP Solution: Add 400 µL of Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the reconstituted standard.

Add 1 mL ultrapure water to the provided lyophilized product (CEA239-C02) , dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted rHuman Sonic Hedgehog Standard is 20 ng/mL.

NOTE: *Avoiding vigorous shaking or vortexing.*

Prepare the standard serial dilutions.

1. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
2. Add 150 µL of the reconstituted rHuman Sonic Hedgehog Standard and 850 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 = 3000 pg/mL).
3. Prepare serial dilutions for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
4. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 1500 pg/mL).
5. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
6. Sample Dilution Buffer serves as zero standard (blank).

IMPOTANT: For BSS matrix samples, do not use the Sample Dilution Buffer in the kit to prepare gradient standards. Instead, prepare 1% (v/v) Tween 20 solution as the sample dilution buffer to prepare gradient standards and the zero concentration of the standard curve.

Preparation the 1% (v/v) Tween 20 solution:

1. BSS buffer should be stored at 2°C~25°C, and Tween 20 reagent should be stored at room temperature. Before preparation, the two reagents should be taken out in advance and balanced to return to room temperature.

2. Take the preparation of 10 mL of 1% (v/v) Tween 20 solution as an example. Measure 9.9mL of BSS solution into a 15 mL centrifuge tube, and then add 0.1 mL of Tween 20 reagent to it. After adding, seal the tube tightly. Use the reverse rotation method and turn it up and down 5 to 10 times to ensure the solution is thoroughly mixed. During this process, try to avoid foaming.
3. After the 1% (v/v) Tween 20 solution is prepared, it can be used as a diluent for the standard and the sample to be tested. It can be stored for one week at 2°C~8°C.

Pretreatment of BSS matrix samples

For BSS matrix samples, an appropriate amount of Tween 20 solution should be added to make the final concentration of Tween 20 in the sample to be tested 1% (v/v) before measurement. For samples that have been added with Tween 20 and need to be diluted, please dilute them with 1% (v/v) Tween 20 solution before measurement.

PROCEDURE OF ASSAY

1. Add 50 µL of rHuman Sonic Hedgehog Standard, sample, or control to wells, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours**.
2. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
3. Add 50 µL Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours**.
4. Repeat step 2.
5. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light**.
6. Repeat step 2.
7. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
8. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.
9. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.

Note: the color in the wells should change from blue to yellow.

Note: To reduce the background noise, subtract the readings at 630nm from the

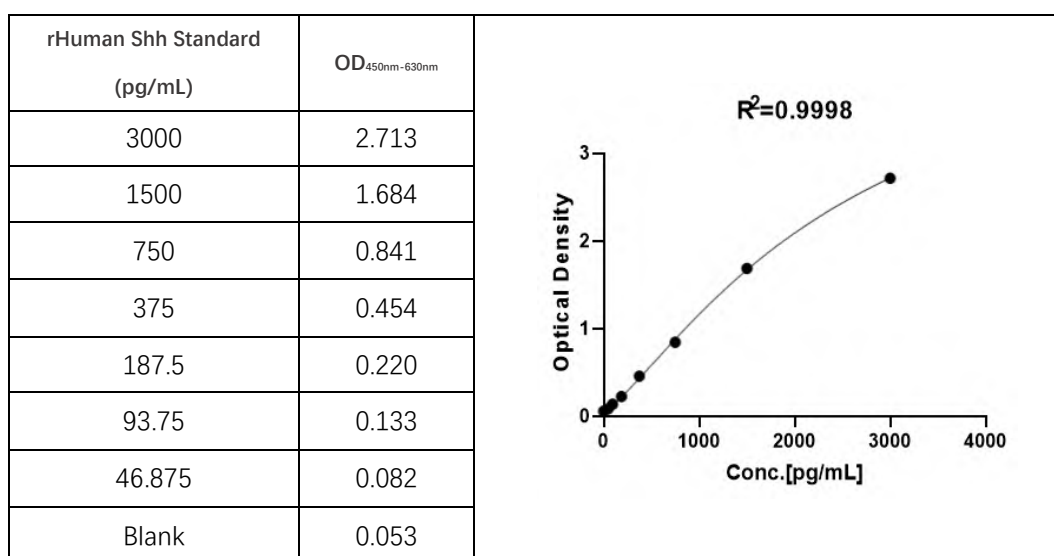
readings at 450nm.

CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.



PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of rHuman Sonic Hedgehog is typically less than 30 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

2. Intra-Assay Precision

Ten replicates of each of 3 samples containing different rHuman Sonic Hedgehog concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
2000	1900.95	46.81	10	2.5
500	474.55	17.87	10	3.8
125	116.35	7.84	10	6.7

3. Inter-Assay Precision

3 samples containing different concentrations of rHuman Sonic Hedgehog were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
2000	1980.15	99.93	9	5.0
500	492.86	25.89	9	5.3
125	120.15	10.84	9	9.0

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts