ClinMax™ Human Insulin ELISA Kit

Catalog Number：CEA-B205

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.

Product information

This kit is specifically designed for the accurate quantitation of human Insulin from cell culture supernates, serum and plasma.

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.

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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.

Manufactured and distributed by
ACRODiagnostics Inc.
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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA205-C01	Pre-coated Anti-Insulin Antibody Microplate	1 plate
CEA205-C02	Human Insulin Standard (Std.-1)	300 μIU/mL
CEA205-C03	Biotin-Anti-Insulin Antibody Con. Solution	400 μL
CEA205-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA205-C05	Streptavidin-HRP Con. Solution	500 μL
CEA205-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA205-C07	20× Washing Buffer	50 mL
CEA205-C08	Sample Dilution Buffer	15 mL×2
CEA205-C09	Substrate Solution	12 mL
CEA205-C10	Stop Solution	6 mL

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 content listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-Insulin 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.

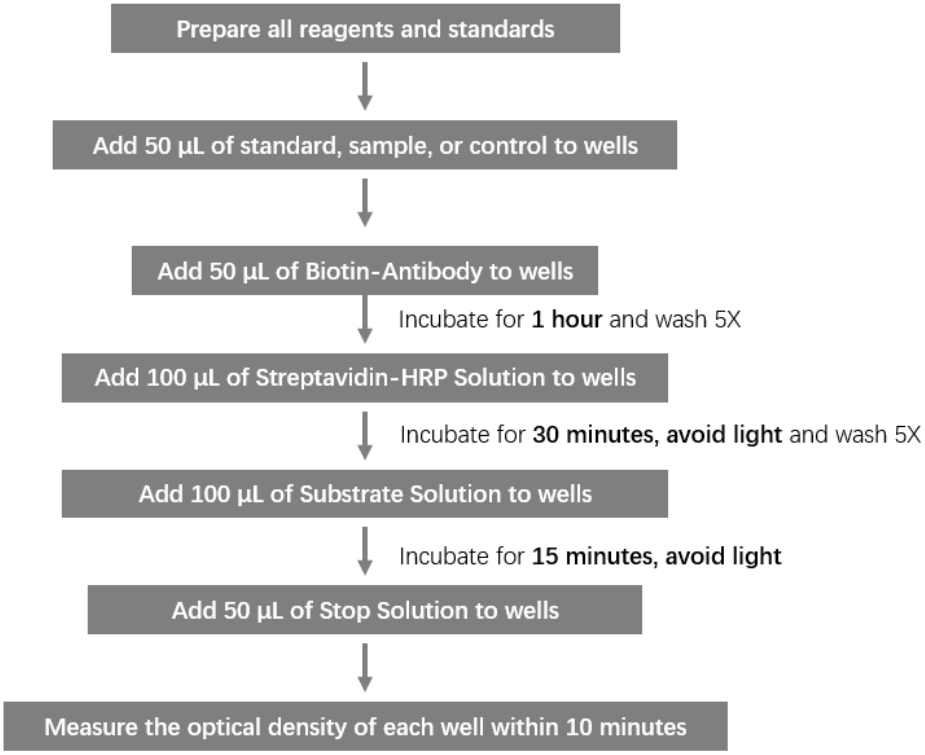
NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte: Insulin



NOTE: Incubation temperature is 18 ℃-25 ℃

Prepare the working buffers and standard dilutions.

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.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin-Anti-Insulin Antibody Solution: Add 240 µL of Biotin-Anti-Insulin Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Insulin Streptavidin-HRP Solution: Add 100 µL of Insulin Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the standard serial dilutions.

1. Label 6 tubes, one for each standard point: Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
2. Add 300 µL of the liquid from **Human Insulin Standard (Std.-1)** and 300 µL of Sample Dilution Buffer to tube Std.-2, thoroughly mix (Std.-2 =150 µIU/mL).
3. Prepare serial dilutions for the standard curve as follows: Add 300 µL of Sample Dilution Buffer to each tube (Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
4. Transfer 300 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 75 µIU/mL).
5. Continue to transfer 300 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7 (4.69 µIU/mL).
6. Sample Dilution Buffer serves as zero standard (blank).

PROCEDURE OF ASSAY

1. Add 50 µL of Insulin Standard, sample, or control to wells.
2. Add 50 µL Biotin-Anti- Insulin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour.**
3. 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.
4. Add 100 µL of Insulin Streptavidin-HRP Solution to each well. Seal the plate with

microplate sealing film. Incubate at room temperature (18-25 °C) for 3**0 minutes, avoid light.**

5. Repeat step 3.
6. 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**.
7. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.

   Note: *the color in the wells should change from blue to yellow.*
8. Read the absorbance at 450nm and 630nm using Microplate reader within 10 minutes.

   Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

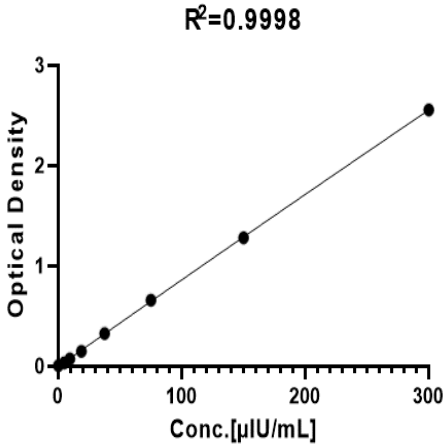
CALCULATION OF RESULTS

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: $R^2 \geqslant 0.9900$.
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.*

Insulin Standard (μIU/mL)	$OD_{450nm-630nm}$
300	2.558
150	1.283
75	0.662
37.5	0.329
18.75	0.151
9.38	0.075
4.69	0.036
Blank	0.008



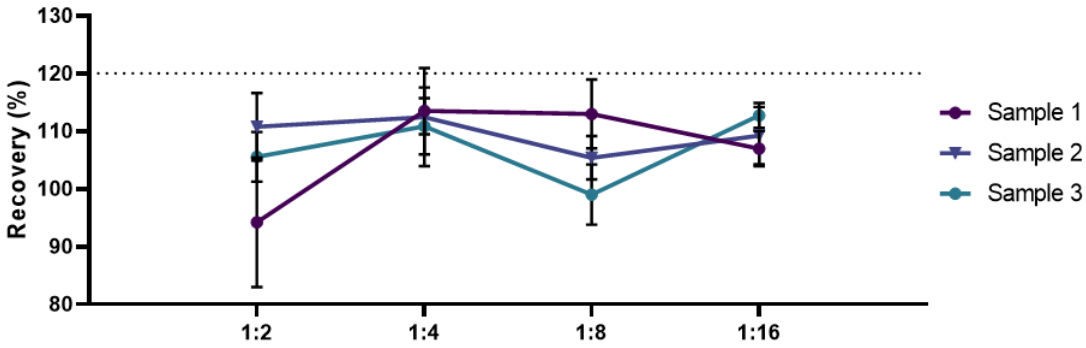
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of Insulin is typically less than 4.69 μIU/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. Linearity

Three samples (Serum) spiked with high concentrations of Insulin were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of Insulin for serum samples is 107.8%.



3. Intra-Assay Precision

Ten replicates of each of 4 samples containing different Insulin concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (μIU/mL)	Mean (μIU/mL)	SD	Numbers	CV(%)
300	302.63	4.87	10	1.6
150	148.63	2.15	10	1.4
37.5	37.29	2.84	10	7.6
9.38	10.07	0.53	10	5.3

4. Inter-Assay Precision

Five samples containing different concentrations of Insulin were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (μIU/mL)	Mean (μIU/mL)	SD	Numbers	CV(%)
300	300.23	0.53	9	0.2
150	149.55	1.02	9	0.7
37.5	36.43	3.31	9	9.1
9.38	9.59	1.06	9	11.1
4.69	6.94	1.01	9	14.5

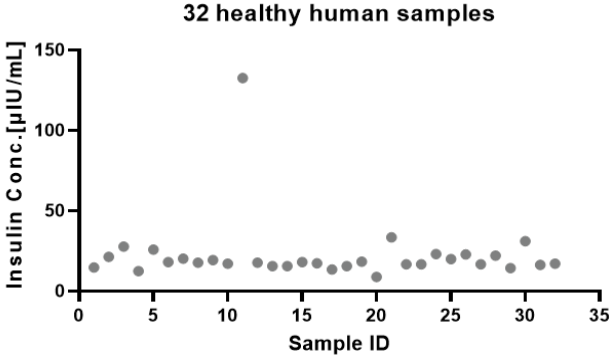
5. Recovery

Recombinant Insulin was spiked into 3 human serum samples, and then analyzed. The average recovery of Insulin for serum samples is 103.8%.

Sample ID	Conc Measured (μIU/mL)	Conc Added(μIU/mL)	Conc Recovered(μIU/mL)	Recovery(%)
1	262.42	225	249.67	111.0
	170.26	150	157.50	105.0
	86.31	75	73.55	98.1
	14.17	-	-	-
2	235.65	225	218.44	97.1
	162.23	150	145.02	96.7
	89.43	75	72.22	96.3
	19.12	-	-	-
3	295.94	225	251.06	111.6
	213.36	150	168.49	112.3
	124.55	75	79.68	106.2
	49.86	-	-	-

6. Sample Values

32 healthy serum samples were evaluated for the concentrations of human Insulin in assay.



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