Human Insulin (INS) ELISA Kit

(Double Antibody Sandwich Method)

96 Wells

Catalogue Number: NE01I0004

Valid Period: 6 months

For samples:

ELISA Kit for the quantitative Measurement of Human Insulin in Serum, Plasma, Body Fluids and Tissue Homogenates

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. BACKGROUND

Insulin is a peptide hormone encoded by the INS gene and synthesized by β-cells of human pancreatic islets. The single-chain proinsulin comprises a 30-amino-acid B chain (aa25-54), a C-peptide segment (aa55-89), and a 21-amino-acid A chain (aa90-110). Proteolytic cleavage of the C-peptide generates biologically active insulin, a disulfide-linked heterodimer consisting of the A and B chains.

As the primary anabolic hormone in the body, insulin regulates carbohydrate, lipid, and protein metabolism by promoting the uptake of blood glucose into hepatocytes, adipocytes, and skeletal muscle cells. Elevated insulin levels in the blood strongly inhibit hepatic glucose production and secretion. Circulating insulin also modulates protein synthesis in various tissues, thereby facilitating the conversion of small circulating molecules into intracellular macromolecules. In contrast, low blood insulin levels induce extensive catabolism, particularly of stored adipose tissue, exerting the opposite effect.

Known insulin-associated disorders include hyperinsulinemia and maturity-onset diabetes of the young type 10 (MODY10). A variety of mutant alleles with phenotypic effects have been identified, which are associated with insulin-dependent diabetes mellitus (IDDM), permanent neonatal diabetes mellitus (PNDM), maturity-onset diabetes of the young type 10 (MODY10), and hyperproinsulinemia.

2. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the double antibody sandwich Enzyme Linked Immunosorbent Assay to detect the concentration of Insulin in samples. The microtiter plate has been pre-coated with anti-Insulin antibody, standards or samples are then added to the microtiter plate wells and Insulin if present, will bind to the antibody pre-coated wells under specific conditions. Then wash the plate to remove unbound substances, add the HRP-conjugated antibody, and incubate to form a double-antibody sandwich complex with the coated antibody, Insulin, and detection antibody. Then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP, and finally turns to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with total Insulin bound in the initial steps. The color is

measured by spectrophotometrically with wavelength of 450nm. The concentration of Insulin in samples is then determined by comparing the O.D. of the samples to the standard curve.

3. KITS' ADVANCEMENT

1) High Specificity: Capture antibody and detection antibody are monoclonal antibodies, which respectively identify different epitopes of the antigen and maximizes the specificity of the reaction. No cross-reactivity among homologous analytes.
2) High Stability: The experiment uses high-quality capture antibodies and antigens, and also applies the broad-spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.

4. MATERIALS (Note: Store at 2-8℃)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500ng/mL	50μL	1 vial
3	HRP-conjugated Detection antibody (100×)	150μL	1 vial
4	TMB Substrates	10mL	1 vial
5	Stop Solution	10mL	1 vial (Avoid Light)
6	Wash Solution (100×)	10mL	1 vial
7	Diluent Buffer (10×)	10mL	1 vial
8	Plate Sealer		4 pieces
9	Instruction Manual		1

5. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000μL Pipettor
2) Multichannel Pipettor
3) 1L Sterilized Ddeionized Water or Ultrapure Water
4) Sterilized EP tubes
5) Absorbent Paper
6) Microplate Reader

7) High-speed Centrifuge

8) Microplate Washer or Washing Bottles

9) Incubator or Water Bath (Optional)

10) Data Analysis and Graphing Software

11) Preparation of PBS (pH 7.4): NaH_2PO_4 0.2g, $Na_2HPO_4 \cdot 12H_2O$ 2.9g, NaCl 8g, KCl 0.2g. Then dilute these to 1000mL with distilled water.

6. SPECIMEN COLLECTION AND STORAGE

Serum - Collect blood without anticoagulant and allow samples to clot for 1-2 hours at room temperature. Centrifuge at 1000×g (or 3000 rpm) for 15 minutes. Collect serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma - Collect plasma with an anticoagulant (eg., EDTA or heparin) at a final concentration of 1%. After collection, allow it to sit at room temperature or 4°C for 30 minutes. Centrifuge at 1000×g (or 3000 rpm) for 15 minutes, collect the supernatant, and store in aliquots at -20°C or -80°C for future use.

Tissue lysate - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, the tissue is first washed with pre-chilled PBS (0.01mol/L, pH7.2-7.4) and then weighed. Typically, 0.3g-0.5g of tissue is added to 500μL of pre-chilled PBS (0.01mol/L, pH7.2-7.4) and homogenized in a glass container on ice. The cell membrane is lysed using ultrasonic treatment or repeated freeze-thaw cycles. After centrifugation at 1500×g (or 5000 rpm) for 15 minutes, collect the supernatant and store in aliquots at -20°C or -80°C for future use.

Cell lysate - Suspension cells can be collected by centrifugation directly. Adherent cells should be detached with trypsin and then collecte by centrifugation. Trypsin digestion should be avoided when measuring certain indicators. Wash cells with PBS (0.01mol/L, pH7.2-7.4) three times. Resuspend the cells in a small volume of PBS, and lyse the cell membrane using ultrasonic treatment or repeated freeze-thaw cycles.fter centrifugation at 1500×g (or 5000 rpm) for 15 minutes, collect the supernatant and store in aliquots at -20°C or -80°C.

Cell culture supernatants - Collect cultured cells, Centrifuge at 1000×g (or 3000 rpm) for 15

minutes, and collect the supernatant. Assay immediately or store samples at -20°C or -80°C.

7. PREPARATION BEFORE ASSAY

1) Please read the kit instruction manual carefully before use. All reactions are performed at room temperature (20-25°C; the same applies hereinafter).
2) Sample Preparation: The frozen samples should be slowly restored to room temperature and then mix well, centrifuge the samples at 12000 rpm for 1 minute. Collect the supernatant for immediate assay.
3) Sample Dilution: Please predict the concentration before assaying. If the predicted concentration of your sample exceeds the maximum detection limit of this kit, it is recommended to dilute the sample with PBS. For the initial assay, multiple dilution factors are recommended, with serial 10-fold dilutions between adjacent concentrations.

NOTE:

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Due to variations in cell stability, cell number, and sampling time, cell culture supernatant samples may not be suitable for detection with this kit.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples, and lead to inaccurate results.
- The optimal pH value of the sample is between 7.2-7.4.

8. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30 minutes before using.
2) The preparation of Wash Solution (1×): Dilute 10mL of Wash Solution (100×) with 990mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.
3) The preparation of Diluent Buffer (1×): Dilute 10mL of Diluent Buffer (10×) with 90mL of deionized water or ultrapure water, and mix thoroughly. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely

dissolved.

9. ASSAY PROCEDURES

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. (Note: The microplate frame can be reused).

   The standard may stick to the tube wall due to transport turbulence. Before using, gently shake it or centrifuge for about 2 seconds.

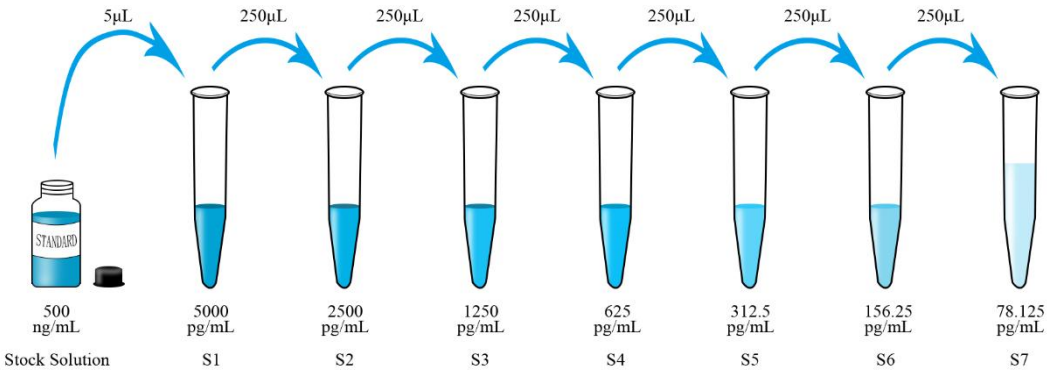
2) Prepare Standards: When preparing the standards, label 7 tubes, S1-S7 and add a certain volume of Diluent Buffer (1×): S1 (495µL), S2 to S7 (each 250µL).

   ① Add 5µL of Standard (Stock Solution - 500ng/mL) into the S1. Mix gently by pipetting up and down twice, then invert to mix thoroughly.

   (All subsequent mixing steps should be performed in the same manner—do not vortex or use any vigorous mixing method.)

   ② Transfer 250µL of the resulting S1 (5000pg/mL) to the tube S2, then transfer 250µL of the resulting S2 solution (2500pg/mL) to S3, mix gently. Continue this 2-fold serial dilution sequentially through S7 to achieve a final concentration of 78.125pg/mL.

   NOTE: There are 7 points of diluted standards, S1: 5000pgng/mL, S2: 2500pgng/mL, S3: 1250pg/mL, S4: 625pg/mL, S5: 312.5pg/mL, S6: 156.25pg/mL, S7: 78.125pg/mL.



3) Sample addition: Add 100µL of standards and samples to be tested into the microplate wells. For the blank control, add only 100µL of sample diluent.

4) Cover the plate with a plate sealer, put it on the oscillator to mix and incubate for 90 minutes at room temperature.

5) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300μL Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times.

  Note: There should be no moisture appears in the fifth washing step.

6) Prepare Detection Antibody: Extract 100μL of Detection antibody (100×), and add it into 9.9mL Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100μL of above diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 90 minutes at room temperature.

7) Washing Step: Repeat the same procedure as step 5.

8) Add 100μL TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15 mins. If the color is light, the reaction time can be extended appropriately, but not more than 30 minutes.

9) Add 50μL of Stop Solution to each well to stop the reaction.

10) Run the microplate reader and conduct measurement at 450nm.

11) Data analysis: Four parameter curve fitting is recommended.

10. NOTES

1) **Sample Preparation**

a) After collection, the samples should be aliquoted and stored at -20°C (less than 3 months) or -80°C (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) Certain chemical lysis buffers (e.g., SDS, Triton, etc.) may interfere with the assay and should be used with caution.

d) It is recommended that all standards, controls and samples be run in duplicate.Gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) Do not use hyperlipidemia or hemolysis samples, which may interfere with ELISA and lead to inaccurate results.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

e) TMB is easily contaminated. please protect it from light and metal.

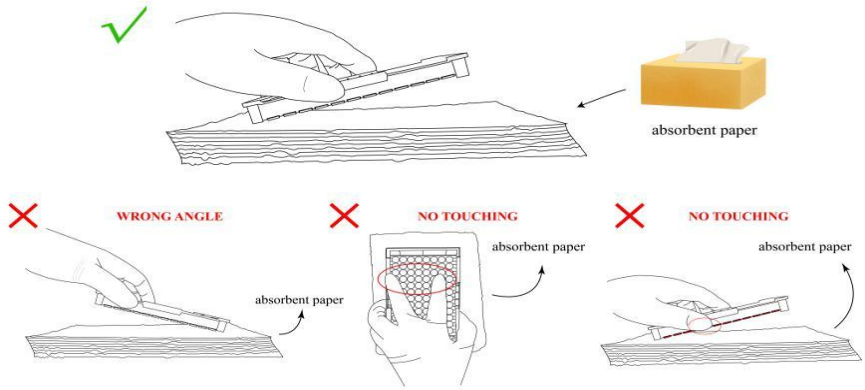
f) Monitor the reaction time. After adding TMB substrate, observe the color change of

reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

i) Schematic diagram of tapping the plate:



11. CALCULATION OF RESULTS

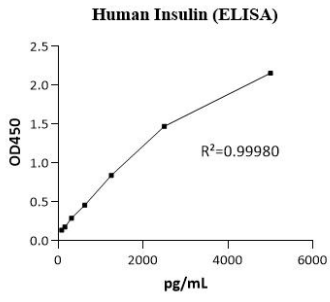
1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.

3) Calculate the concentration of Insulin in the samples corresponding to the mean absorbance from the standard curve.

4) Standard curve for demonstration only.

APPENDIX 1: EXAMPLE OF STANDARD CURVE



	Con pg/mL	OD450
S1	5000	2.158
S2	2500	1.473
S3	1250	0.843
S4	625	0.460
S5	312.5	0.292
S6	156.25	0.179
S7	78.125	0.139

12. QUALITY CONTROL

1) Intra Variation%: 4-9

   Inter Variation%: 2-7

2) Recovery (%):

Spike Concentration	Recovery (%)
Serum	70-130
Plasma	70-130
Cell supernatant	80-120
MCF-7 Complete Culture Medium	85-120

3) Linearity：

Dilution	Range (%)
1:2	94-110
1:4	98-105
1:8	98-103
1:16	97-106
1:32	96-102
1:64	98-103

4) Sensitivity:

   LOD: 19.5pg/mL

   LOQ: 78.125pg/mL

5) Specificity/Cross-Reactivity:

Sample	Cross Reactivity (%)
TfR	NA
IL-8	NA
FGF	NA
IL-6	NA
IGF1-LR3 NA	NA

13. FAQs & TROUBLESHOOTING

If the experimental results are abnormal, promptly take photos to record the color development results, fully retain the unused strips and reagents, and contact technical support. In addition, you may refer to the troubleshooting information provided below to identify the root cause of the problem.

Problem Description	Possible Causes	Corresponding Solutions
Poor Standard Curve	Incorrect dilution of standards	Dilute the standard curve according to the specified ratio.
	Inaccurate pipetting or sample loading	Check the pipette and pipette tips.
	Incomplete washing of the microplate	Ensure the required number of washing cycles and the volume of washing solution per well.
Weak or Absent Color Development	Insufficient incubation time	Ensure adequate incubation time.
	Incorrect experimental temperature	Use the recommended incubation temperature.
	Insufficient reagent volume or missing addition	Check the pipetting and sample loading process to ensure all reagents are added in sequence with sufficient volume.
	Substrate solution not equilibrated to room temperature	Allow the TMB substrate to stand at room temperature for more than 30 minutes before color development.
Low OD Value Reading	Incorrect microplate reader settings	Verify the wavelength and filter configuration on the microplate reader. Preheat the microplate reader in advance before reading.
High Coefficient of Variation (CV)	Incorrect sample loading	Check the sample loading operation.
	Contamination on the microplate bottom	Inspect the microplate bottom for residual liquid and fingerprints.
	Foreign objects or air	Confirm no foreign objects in wells before

	bubbles in wells	sample loading and no air bubbles after loading.
	Unsealed or incompletely sealed plate during incubation	Seal the plate with plate sealing film.
High Background Value	Incomplete washing of the microplate	Wash the plate according to the method recommended in the instruction manual.
		If using an automated plate washer, check for clogs in all liquid inlets and waste outlets.
		For manual washing, appropriately increase the number of washing cycles.
		Inadequate or missed washing will result in high background.
	Incorrect incubation time or temperature	Operate strictly in accordance with the instruction manual.
	Contaminated consumables	Ensure the tubes, pipette tips and other consumables are clean.
	Contaminated washing solution	Prepare fresh washing solution.
	Contaminated substrate solution	The substrate solution is inherently colorless. Ensure the substrate is not contaminated by metal ions or oxidizing agents before use and store it away from light.
Low Sensitivity	Improper kit storage	Store all relevant reagents according to the requirements in the instruction manual.

14. CONTACT US

Address: 201318, Building 6, Lane 889, Ziping Road, Zhoupu Town, Pudong New Area, Shanghai, China

Website: www.bluegene.cc www.elisakit.cc

Tel: 400-882-6373/021--61106433/021--61106434/021-61106435/021--61106436

International Marketing Department:		
America	Tina	americas@bluegene.cc
America	Luyuan	luyuan@bluegene.cc
Asia &Australia	Diana	asia@bluegene.cc
Europe & Africa	Raina	europe-raina@bluegene.cc
Others	Cheng	sales@bluegene.cc
Technical Support Department:		
Menan	tech@bluegene.cc	