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ab100578 Insulin Human ELISA Kit

For the quantitative measurement of human Insulin and Proinsulin in serum, plasma, cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's Human Insulin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human Insulin and Proinsulin in serum, plasma, cell culture supernatants.

This assay employs an antibody specific for Human Insulin coated on a 96-well plate. Standards and samples are pipetted into the wells and Insulin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human Insulin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated Streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Insulin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add standard or sample to appropriate wells.

Incubate at room temperature.



Wash and add prepared biotin antibody to each well. Incubate at room temperature.



Wash and add prepared Streptavidin Solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature



Add Stop Solution to each well. Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

The kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. **For extended storage, it is recommended to store at -80°C .**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition	Storage (After preparation)
Insulin Microplate (12 x 8 wells)	96 wells	-20°C	1 month at 4°C*
20X Wash Buffer	25 ml	-20°C	1 month at 4°C
Assay Diluent A	30 ml	-20°C	N/A
5X Assay Diluent B	15 ml	-20°C	1 month at 4°C
Biotinylated anti-Human Insulin	2 vials	-20°C	5 days at 4°C
Recombinant Human Insulin Standard	2 vials	-20°C	1 week at -80°C
500X HRP-Streptavidin Concentrate	200 µl	-20°C	Do not store and reuse.
TMB One-Step Substrate Reagent	12 ml	-20°C	N/A
Stop Solution	8ml	-20°C	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- **Do not vortex the standard during reconstitution, as this will destabilize the protein.**
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- Make sure you have the right type of plate for your detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent B

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 ml of 20X Wash Solution Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Solution.

9.3 1X Biotinylated Insulin Detector Antibody

Briefly spin the Biotinylated anti-Human Insulin vial before use. Add 100 µl of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 80-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 500X HRP-Streptavidin concentrate vial before use. HRP-Streptavidin concentrate must be diluted 500-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 30 µl of 500X HRP-Streptavidin concentrate into a tube with 15 ml 1X Assay Diluent B to prepare a final 500-fold diluted 1X HRP-Streptavidin solution. Mix well.

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- Standard (recombinant protein) should be stored at -20°C or 80°C (recommended at -80°C) after reconstitution.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Briefly spin the vial of Recombinant Human Insulin Standard. Prepare a 1400 $\mu\text{U/ml}$ Insulin Stock Standard by adding 400 μl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into the Insulin Standard vial. Mix thoroughly and gently.

10.2 Label tubes #1 – 7.

10.3 Prepare **Standard #1** by adding 150 μl 1400 $\mu\text{U/ml}$ **Stock Standard** to 550 μl Assay Diluent A or 1X Assay Diluent B into tube 1#. Mix thoroughly and gently.

10.4 Pipette 300 μl Assay Diluent A or 1X Assay Diluent B into each tube.

10.5 To prepare **Standard #2**, add 300 μL of the **Standard #1** into tube #2 and mix gently.

10.6 To prepare **Standard #3**, add 300 μL of the **Standard #2** into tube #3 and mix gently.

10.7 Using the table below as a guide, prepare subsequent serial dilutions. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 $\mu\text{U/ml}$).

Standard #	Volume to dilute (μL)	Diluent (μL)	Total Volume (μL)	Starting Conc. (μIU/mL)	Final Conc. (μIU/mL)
1	150	550	700	1400	300
2	300	300	600	300	150
3	300	300	600	150	75
4	300	300	600	75	37.5
5	300	300	600	37.5	18.75
6	300	300	600	18.75	9.38
7	300	300	600	9.38	4.69
8 (Blank)	0	300	300	0	0

11. Sample Preparation

- For serum and plasma samples dilutions: use Assay Diluent A
- For cell culture supernatant samples dilution: use 1X Assay Diluent B
Note: If Assay Diluent A is used by mistake on cell culture supernatant samples, this may affect the results and give no signal
- Suggested dilution for normal serum/plasma: 2-10 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all standards, controls and samples in duplicate.
- 13.1** Add 100 µl of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
 - 13.2** Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1X Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
 - 13.3** Add 100 µl of 1X Biotinylated Insulin Detection Antibody (Reagent Preparation section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.
 - 13.4** Discard the solution. Repeat the wash as in step 13.2.
 - 13.5** Add 100 µl of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
 - 13.6** Discard the solution. Repeat the wash as in step 13.2.
 - 13.7** Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
 - 13.8** Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.
 - 13.9** Analyze the data as described below.
 - 13.9.1** Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density.
 - 13.9.2** Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.
 - 13.9.3** Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

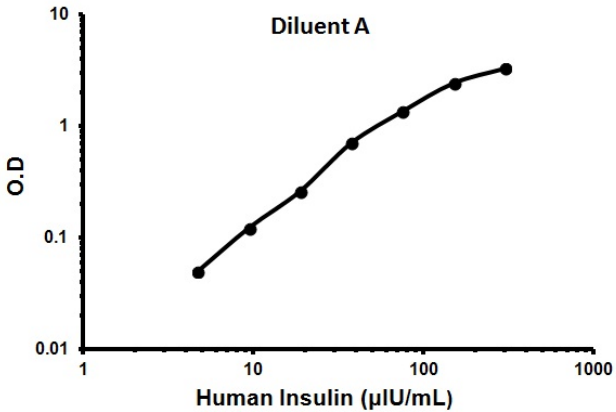


Figure 1. Example of Insulin standard curve in Diluent A. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

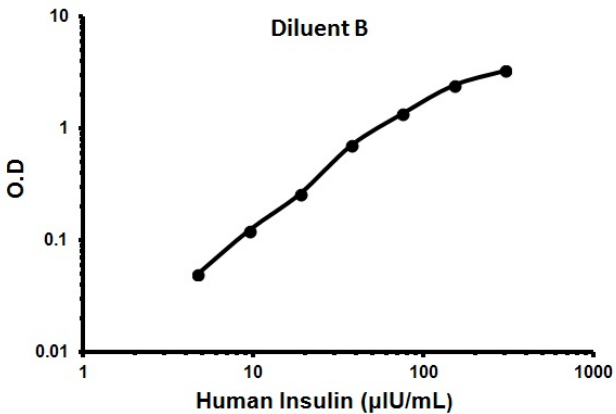


Figure 2. Example of Insulin standard curve in Diluent B. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Conc. (μ U/ml)	O.D.	
	Assay Diluent A	Assay Diluent B
4.688	0.050	0.050
9.375	0.122	0.122
18.75	0.259	0.259
37.5	0.701	0.701
75	1.347	1.347
150	2.415	2.415
300	3.265	3.265

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Insulin is typically less than 4 μ U/ml.

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

RECOVERY –

Recovery was determined by spiking various levels of Human Insulin into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	91.40	83-102
Plasma	99.03	73-128
Cell Culture Media	76.16	68-88

Linearity of Dilution

Serum Dilution	Average % Expected Value	Range (%)
1:2	100.2	90-108
1:4	122.1	112-135

Plasma Dilution	Average % Expected Value	Range (%)
1:2	109.5	102-128
1:4	131.7	121-140

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	86.69	72-103
1:4	82.43	68-90

16. Assay Specificity

This kit recognizes Insulin and Proinsulin.

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Human BDNF, BLC, ENA-78, IL-1 α , IL-1 β , IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN- γ , Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1 α , MIP-1 β , MIP-1 δ , PARC, PDGF, RANTES, SCF, TARC, TGF- β , TIMP-1, TIMP-2, TNF- α , TNF- β , TPO, VEGF.).

17. Species Reactivity

This kit recognizes human Insulin and Proinsulin.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C , all other assay components 4°C . Keep substrate solution protected from light.
	Stop solution	Stop solution should be added to each well before measure.

19. Notes

Technical Support

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