Human Insulin ELISA Kit

Catalog no. KAQ1251

Pub. No. MAN0004817 **Rev** 4.0

Description

The Human Insulin (Hu Insulin) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of human insulin in human serum.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. no. KAQ1251 96 tests	Color Code	Reconstitution
Antibody Coated Wells. 96 well plate.	1 plate	Blue	Ready to use.
Standard 0 µL/mL in human plasma, contains thymol; lyophilized.	1 vial	Yellow	Add 2 mL distilled water.
Standards 1 to 5 in human serum, contains thymol; lyophilized. Refer to vial label for quantity and reconstitution volume.	5 vials	Yellow	Add 1 mL distilled water.
Controls 1 and 2 in human serum, contains thymol. Refer to label or ranges; lyophilized.	2 vials	Silver	Add 1 mL distilled water.
Wash Buffer Concentrate (200X).	10 mL	Brown	Dilute 1 mL in 199 mL distilled water.
Anti-insulin HRP Conjugate. Contains thymol.	6 mL	Red	Ready to use.
Stabilized Chromogen, Tetramethylbenzidine (TMB).	12 mL	Orange	Ready to use.
Stop Solution, 1.0 N HCl.	12 mL	White	Ready to use.

Note: Standard 0 µIU/mL is recommended for sample dilutions.

Note: One µIU of standard preparation is equivalent to 1 µIU MRC 2nd IRP 66/304.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/techresources** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (200X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 1 mL of Wash Buffer Concentrate (200X) with 199 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Discard unused 1X Wash Buffer at the end of the day.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- Samples may be stored for 24 hours at 2-8°C

Dilute samples

- No special pretreatment of the sample is necessary. Samples suspected to contain insulin concentrations greater than the highest standard are to be diluted with the zero standard.
- Prior to use, all samples should be at room temperature. Vortex the samples before use.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards and controls

Note: Use glass or plastic tubes for diluting standards.

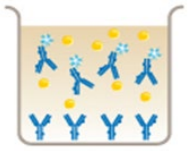
1. Reconstitute standards and controls to the volume specified on the vial label with distilled water (2 mL for the zero standard, 1 mL for the other standards, and 1 mL for controls). Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion. After reconstitution, standards and controls should be aliquoted and stored at –70°C. Avoid successive freeze-thaw cycles. **Use the standard within 1 hour of reconstitution**.
2. Discard remaining reconstituted standard or freeze in aliquots at –80°C for up to 4 months. Standard can be frozen and thawed one time only without loss of activity. Return Standard Diluent Buffer to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 45 minutes.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



Bind antigen

1. Add 50 µL standards, samples or controls to the appropriate wells.

Add Anti-Insulin HRP

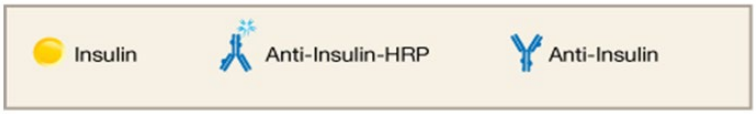
2. Add 50 µL Anti-Insulin HRP conjugate (see page 2) into each well except the chromogen blanks.
3. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
4. Thoroughly aspirate the solution from the wells and wash wells 3 times with 1X Wash Buffer.

Add chromogen

5. Add 100 µL Stabilized Chromogen to each well within 15 minutes following the washing step.
6. Cover the plate with the plate cover and incubate for 15 minutes at room temperature **in the dark**.
   Note: TMB should not touch aluminum foil or other metals.

Add stop solution

7. Add 100 µL of Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.



Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 1 hour after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than that of the highest standard in zero standard and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0-250 µIU/mL Hu insulin.

Standard Hu Insulin (µIU/mL)	Optical Density (450 nm)
250	2.34
128	1.31
44.4	0.51
13.8	0.13
5.1	0.07
0	0.03

Sensitivity

The analytical sensitivity of this assay is 0.17 µIU/mL of Hu insulin as determined by adding two standard deviations to the mean O.D.

Performance characteristics, continued

Intra-assay precision

Samples of known Hu Insulin concentration were assayed in replicates of 23 to determine precision within an assay.

Parameters	Sample 1	Sample 2
Mean (µIU/mL)	13.09	32.9
SD	0.6	1.9
%CV	4.8	6.0

SD = Standard Deviation; CV = Coefficient of Variation

Specificity

The cross-reactivities were determined by addition of different analytes to a serum containing 4 ng/mL (100 µIU/mL) insulin and measuring the apparent insulin concentration. As shown below, animal insulins (except rat insulin) cross-react, whereas human, pork and beef proinsulins present no cross reaction.

Added analyte to a high-value serum (4 ng/mL)	Observed insulin value (ng/mL)	Cross-reaction (%)
Porcine insulin 8 ng/mL	17.4	>100
Bovine insulin 8 ng/mL	17.8	>100
Dog insulin 16 ng/mL	17.2	81
Rabbit insulin 16 ng/mL	14.1	62
Rat insulin 16 ng/mL	3.7	0
Human proinsulin 32 ng/mL	4.4	0.3
Procine proinsulin 16 ng/mL	4.7	2.5
Bovine proinsulin 16 ng/mL	4.4	0.6

High Dose Hook Effect

A sample spiked with Hu Insulin up to 10,000 µIU/mL gives a result higher than the last standard point.

Inter-assay precision

Samples were assayed 8 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2
Mean (pg/mL)	13.29	34.12
SD	1.08	3.10
%CV	8.1	9.0

Linearity of dilution

Sample	Dilution	Theoretical concentration (µIU/mL)	Measured concentration (µIU/mL)
Serum 1	1/1	—	82.3
	1/2	41.2	42.21
	1/4	20.6	22.86
	1/8	10.3	11.04
	1/16	5.15	5.9
	1/32	2.58	3.3
Serum 2	1/1	—	57.5
	1/2	28.7	27.7
	1/4	14.4	14.5
	1/8	7.2	8.0
	1/16	3.6	4.4
	1/32	1.8	2.3

Recovery

Sample	Added INS (µIU/mL)	Recovered INS (µIU/mL)	Recovery (%)
Serum	182.1	174.5	96
	86.7	80	92
	39.6	37.4	94
	15.1	13.6	90

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

For support visit thermofisher.com/support or contact techsupport@lifetech.com.

thermofisher.com

04 November 2022