Insulin ELISA Kit

Catalog Number EIAINSC (96 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Insulin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of insulin in serum, plasma, and tissue culture media. The assay recognizes both natural and recombinant insulin. Characterization of this ELISA kit was done primarily on human samples, but cross-reactivity with bovine and porcine samples is observed. Little or no reactivity is observed with rat or mouse samples.

Human insulin is a 51 amino acid anabolic peptide-hormone secreted by the pancreatic β-cells in the Islets of Langerhans. Insulin exists primarily as a monomer at low concentrations (~10^{-6} M). It dimerizes at higher concentrations at neutral pH, and forms hexameric complexes at high concentrations in the presence of zinc ions.

Contents and storage

Kit and components are shipped at –20℃. Upon receipt, store the kit at –20℃. Once open, store the kit at 4℃ and use within 2 weeks.

Components	Quantity
Insulin Standard; 64,000 pg/mL recombinant human insulin	40 µL
Assay Buffer Concentrate (5X)	28 mL
Insulin Antibody Coated Wells, 96-well strip-well plate	1 plate
Insulin Conjugate	5 mL
Wash Buffer Concentrate (20X)	30 mL
Tetramethylbenzidine (TMB) Substrate	11 mL
Stop Solution; contains 1 M HCl, **CAUSITC**	5 mL
Plate Sealer	2

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Orbital shaker

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.
- Set the orbital shaker to a speed providing optimum agitation without the liquid splashing onto the lid. For an orbital shaker with a 3-mm orbital radius, a speed between 500 and 600 rpm is recommended.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- 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. Thaw completely and mix well (do not vortex) prior to analysis.
- 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.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within 2 hours of dilution.

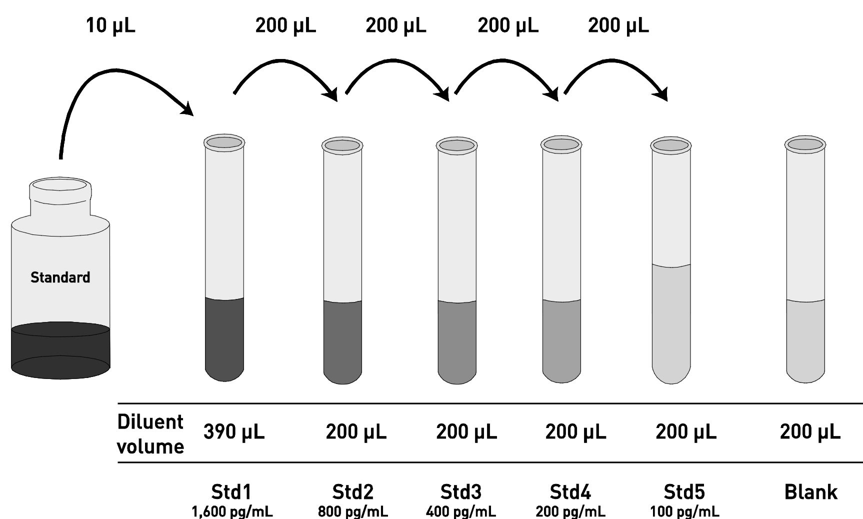
- Dilute **serum and plasma** samples ≥1:5 in 1X Assay Buffer.
- Dilute **tissue culture media** samples ≥1:4 with the corresponding cell culture medium.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Important: For **tissue culture media** samples, **dilute standards with the appropriate tissue culture medium** instead of 1X Assay Buffer.

1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 10 µL Insulin Standard to one tube containing 390 µL 1X Assay Buffer and label as 10 pg/mL human insulin.
3. Add 200 µL Standard Diluent Buffer to each of 5 tubes labeled as follows: 800, 400, 200, 100, and 0 pg/mL human insulin.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **Use the standards within 2 hours of preparation**.

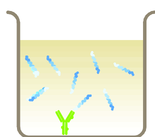


Perform ELISA (Total assay time: 2.0 hours)

IMPORTANT! Perform a standard curve with each assay.

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

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 desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.

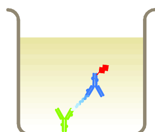


Bind antigen

a. Add 50 µL of standards or samples (see "Pre-dilute samples" on page 2) to the appropriate wells.

b. Cover the plate with plate sealer and incubate for 60 minutes at room temperature on an orbital shaker.

c. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.

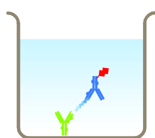


Add detection antibody

a. Add 50 µL Insulin Conjugate into each well.

b. Incubate for 30 minutes at room temperature on an orbital shaker.

c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 300 µL of 1X Wash Buffer.

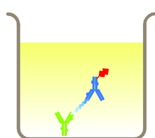


Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.

b. Incubate for 30 minutes at room temperature.

Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL 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 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 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 1X Assay Buffer or the appropriate tissue culture medium and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data was obtained for the various standards over the range of 0–1,600 pg/mL human insulin.

Standard Hu Insulin (pg/mL)	Optical Density (450 nm)
1,600	1,493
800	0.600
400	0.264
200	0.138
100	0.110
0	0.081

Inter-assay precision

Samples were independently run five times in twenty assays in duplicate to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,019.1	678.4	374.5
%CV	11.5	12.0	11.2

CV = Coefficient of Variation

Intra-assay precision

Samples of known human insulin concentration were assayed in replicates of ≥18 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,013.5	665.3	371.8
%CV	1.5	3.2	2.4

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

A number of human serum samples diluted 1:5 to 1:40 in 1X Assay Buffer were tested with the kit.

Sample	Range (pg/mL)	Average (pg/mL)
Serum	982–4,253	1,889

Linearity of dilution

Linearity was determined by assaying two diluted human serum samples (high sample 1,292.5 pg/mL insulin; low sample 542.0 pg/mL insulin), mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80	20	1,142.4	1,169.3	102.4
60	40	992.3	947.7	95.5
40	60	842.2	756.4	89.8
20	80	692.1	590.7	85.3
			Mean Recovery	93.3%

Sensitivity

The analytical sensitivity of the assay is 48.8 pg/mL human insulin. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 22 times, and calculating the corresponding concentration.

Specificity

The following samples were tested using the assay and cross-reactivity calculated within the standard curve.

Sample	% Reactivity
Human insulin	100
Porcine insulin	301.9
Bovine insulin	267.7
Human glucagon	0.03
Human C peptide	0.03
Human proinsulin	<0.01
Rat insulin	<0.01

Limited product warranty

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