Product Manual

# Insulin ELISA kit

Catalog #: ENZ-KIT141-0001

96-Well Kit

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Product Manual

Please read entire booklet before proceeding with the assay.

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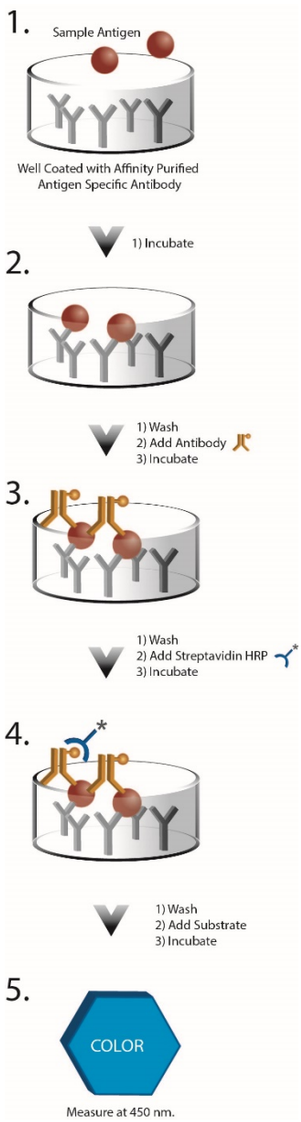
## INTRODUCTION

The Insulin ELISA kit is a complete kit for the quantitative determination of Insulin in Serum, Plasma, and Tissue Culture media. Please read the complete kit insert before performing this assay.

Insulin is peptide hormone made in the pancreas by beta cells. It promotes the uptake of glucose from the blood into tissues where it is stored in the form of glycogen and fat. Insulin also inhibits the production of glucose by the liver[1]. By controlling glucose levels, insulin serves as the central regulator of fat and carbohydrate metabolism. The disease diabetes mellitus results when control of glucose levels is lost due to lack of insulin production (type 1 diabetes) or when sufferers develop an inability to respond to insulin (type 2 diabetes)[2]. Understanding a patient's insulin level helps to differentiate which form of diabetes may be present in patients presenting with diabetic symptoms. Insulin immunoassays assist in identifying insulin resistance in type 2 diabetics and can indicate when insulin supplementation may be necessary in addition to oral medications. Insulin resistance has been linked to a variety of disease states including kidney damage and metabolic syndrome. Insulin immunoassays also help diagnose the presence of an insulin-producing tumor in the islet cells of the pancreas (insulinoma) and can also help determine the cause of low blood glucose (hypoglycemia)[1,3]. Beyond insulin's ability to induce widespread systemic effects, recent studies have demonstrated more localized insulin signaling with impacts on stem cell proliferation[4].




## PRINCIPLE

1. The kit uses a monoclonal antibody to insulin immobilized on a microtiter plate. Standards or samples containing Insulin are added to the plate.
2. The plate is then incubated. During this incubation the insulin antibody immobilized on the plate binds insulin in the standards or samples.
3. The plate is washed, removing excess unbound sample or standard. A solution of biotinylated monoclonal antibody to insulin is then added. This antibody binds to the insulin captured on the plate.
4. After a short incubation the plate is washed to remove unbound biotinylated antibody. Streptavidin conjugated to horseradish peroxidase (SA-HRP) is added and the plate is then incubated.
5. The plate is washed to remove excess conjugate. TMB substrate solution is added to all wells and incubated. An HRP-catalyzed reaction generates a blue color in the solution.
6. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of insulin in the sample or standard.

## MATERIALS SUPPLIED

Do not mix components from different kit lots or use beyond the expiration date of the kit.

1. **Insulin Microtiter Plate, One Plate of 96 Wells**
   **Catalog No. 80-2737**

   A plate using break-apart strips coated with a monoclonal antibody specific to insulin

2. **Insulin Standard, 50 ng/mL**
   **Catalog No. 80-2736**

   One vial containing 100 µL of 50 ng/mL recombinant insulin

3. **Insulin Antibody**
   **Catalog No. 80- 2738-0120**

   One vial containing 120 µL biotinylated insulin monoclonal antibody

4. **Insulin Conjugate, 10 mL**
   **Catalog No. 80-2739-0010**

   A solution of Streptavidin conjugated to horseradish peroxidase

5. **Assay Buffer 13, 60 mL**
   **Catalog No. 80-1500**

   Tris buffered saline containing BSA and detergents

Protect substrate from prolonged exposure to light.

6. **TMB Substrate, 10 mL**
   **Catalog No. 80-0350**

   A solution of 3,3’5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide

Stop solution is caustic. Keep tightly capped.

7. **Stop Solution 2, 10 mL**
   **Catalog No. 80-0377**

   One bottle containing 1N hydrochloric acid in water

8. **Wash Buffer Concentrate, 100 mL**
   **Catalog No. 80-1287**

   One bottle containing 20x Tris buffered saline containing detergent

9. **Insulin Assay Layout Sheet**
   **Catalog No. 30-0341**

10. **Plate Sealer, 3 each**
    **Catalog No. 30-0012**

## STORAGE

All kit components are stable at 4°C until the kit’s expiration date. Shipping conditions may not reflect storage conditions.

## OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. A microplate shaker
7. Adsorbent paper for blotting
8. Microplate reader capable of reading a 450 nm
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

If buffers other than those provided are used, the end-user must determine the appropriate dilution and assay variation.

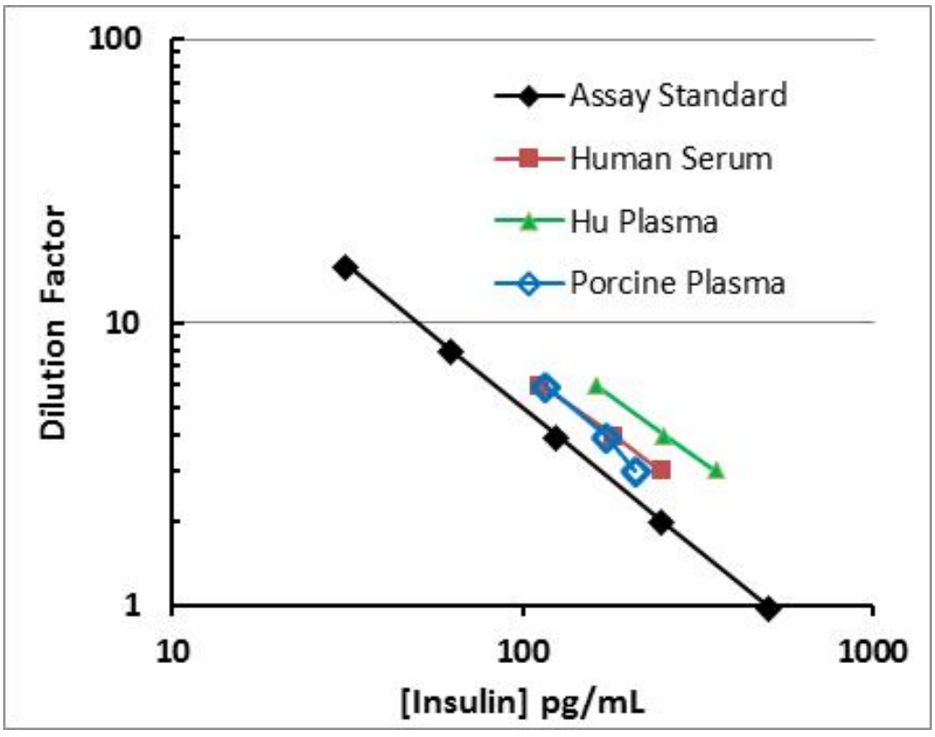
## SAMPLE HANDLING

The Insulin ELISA is compatible with human serum, human plasma, porcine plasma and tissue culture media. Samples diluted sufficiently into Assay Buffer 13 can be read directly from a standard curve. The recommended minimal dilution to remove matrix interference for serum and plasma samples is 1:3 with Assay Buffer 13. Tissue culture media (TCM) can be tested diluted 1:2 with Assay Buffer 13. The minimal recommended dilution may not be optimal for all samples as the levels of endogenous insulin can vary between sample groups. Therefore, it is up to each end user to optimize the dilution for their unique set of samples.

## SAMPLE MATRIX PROPERTIES

### Parallelism and Dilutional Linearity

The parallelism of various matrices was determined by running serial dilutions in the assay, assigning concentrations to each dilution and plotting the dilution factor against the determined concentration of each matrix dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human and canine origin.




Dilutional linearity in matrix dilutions is achieved when returned values approximately match after multiplying them by the dilution factor. Linearity was achieved at 1:3 for human serum, human plasma and porcine serum and at 1:2 for TCM.

| Dilutional Linearity | | | |
|---|---|---|---|
| Dilution Factor | Human Serum | Human EDTA Plasma | Porcine Serum |
| 3 | 100% | 100% | 100% |
| 4 | 98% | 95% | 110% |
| 5 | 78% | 83% | 97% |
| 6 | 90% | 91% | 110% |

| Dilutional Linearity | |
|---|---|
| Dilution Factor | Tissue Culture Media |
| 2 | 100% |
| 3 | 94% |
| 4 | 101% |

### Spike and Recovery

Insulin was spiked at three concentrations into diluted human serum, EDTA plasma and tissue culture media. Matrix background was subtracted and the percent recovery of the spiked insulin was determined.

| Sample Matrix | Dilution | Spike Concentration (pg/mL) | % Recovery of Spike |
|---|---|---|---|
| Human Serum | 1:3 | 500 | 89 |
| | | 250 | 96 |
| | | 125 | 106 |
| Human Plasma | 1:3 | 500 | 77 |
| | | 250 | 80 |
| | | 125 | 98 |
| Tissue Culture Media | 1:2 | 250 | 125 |
| | 1:3 | 125 | 118 |
| | 1:4 | 62.5 | 120 |

## REAGENT PREPARATION

Sample handling procedures should be completed prior to reagent preparation.

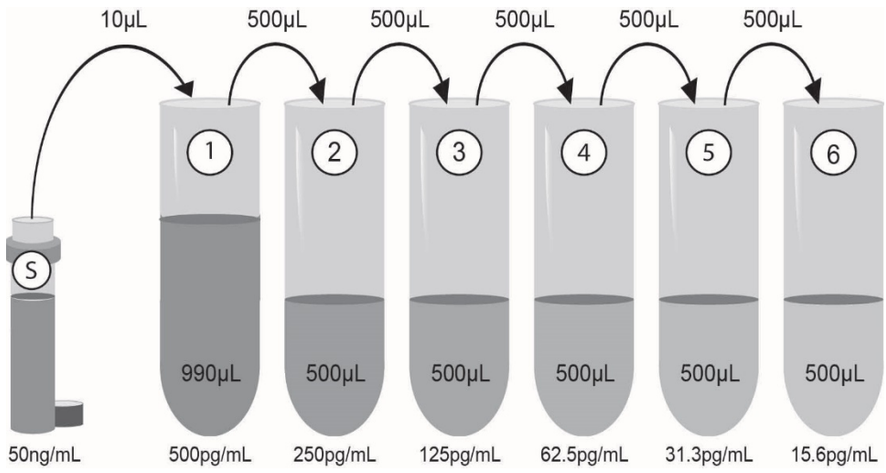
Polypropylene tubes may be used for standard preparation. Avoid polystyrene.

### 1. Wash Buffer

Prepare Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. Store the diluted wash buffer at room temperature. Diluted wash buffer should be used within 3 months.

### 2. Insulin Standard

Allow the insulin standard to warm to room temperature. Label six 12x75 mm polypropylene tubes #1 through #6. Add 990 µL of Assay Buffer 13 into tube #1. Add 500 µL of Assay Buffer 13 into tube #2 through tube #6. Add 10 µL of 50 ng/mL insulin standard stock to tube #1 and vortex. Add 500 µL of tube #1 into tube #2 and vortex. Add 500 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.




**Diluted standards should be used within 60 minutes of preparation. Discard any unused standard dilutions.**

**All other kit components should be brought to room temperature prior to use in the assay.**

### 3. Insulin Detector Antibody

Dilute the Insulin Detector Antibody to working concentration by preparing a 1:100 dilution of the provided stock solution in Assay Buffer 13. For example if 5 mL of detector antibody solution required, add 50 µL of the Insulin Detector Antibody to 4.95 mL of Assay Buffer 13. Store any unused diluted antibody at -20°C. Allow no more than three freeze-thaw cycles.

## ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

All standards and samples should be run in duplicate.

1. Add 100 µL of Assay Buffer 13 into the S0 (0 pg/mL standard) and NSB wells. Leave the TA (Total Activity – enzyme activity control) wells empty.
2. Add 100 µL of standards #1 through #6 into the appropriate wells.
3. Add 100 µL of the samples into the appropriate wells.
4. Seal the plate and incubate at room temperature (RT) on a plate shaker for 60 minutes at ~ 500 rpm*. **See note.**
5. Empty the contents of the wells and wash by adding a full well volume ~400 µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

Add the reagents to the sides of the wells to avoid possible contamination.

6. Add 100 µL of diluted Insulin Detector Antibody into each well, except the NSB and TA wells. Add 100 µL Assay Buffer 13 into NSB wells and leave the TA wells empty.
7. Seal the plate and incubate for 60 minutes with shaking on a plate shaker at room temperature.
8. Wash as above (Step 5).
9. Add 100 µL of Insulin Conjugate to each well.
10. Seal the plate and incubate for 30 minutes with shaking on a plate shaker at room temperature
11. Wash as above (Step 5).
12. Dilute Insulin Conjugate 1:100 with Assay Buffer 13 and add 5 µL of this dilution to TA wells.
13. Add 100 µL of TMB solution into all wells.
14. Seal the plate and incubate for 30 minutes with shaking on a plate shaker at room temperature.
15. Add 100 µL of Stop Solution into each well.
16. Read optical density at 450 nm. If required, the plate reader may be blanked against substrate prior to reading.

*** <u>Note:</u> The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

## CALCULATION OF RESULTS

Be sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of insulin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. The concentration of insulin can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Using data analysis software, plot the Average Net OD for each standard versus insulin concentration in each standard.

## TYPICAL RESULTS

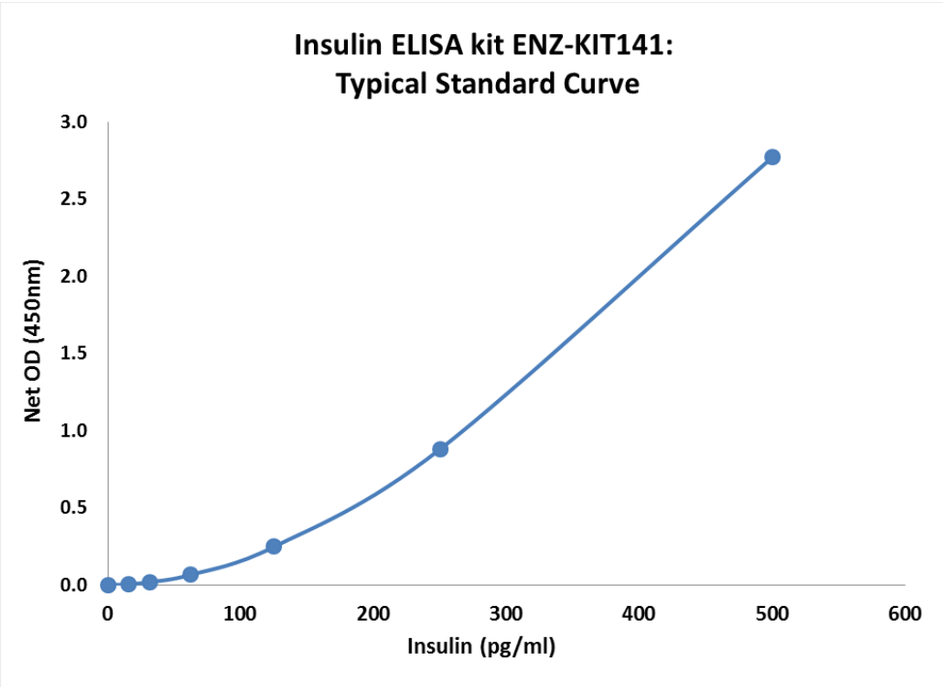
The results shown below are for illustration only and should not be used to calculate results.

| Typical Results | | | | |
|---|---|---|---|---|
| Sample | Mean OD | Net OD | Insulin pg/mL | µIU/mL* |
| TA | 0.645 | 0.457 | -- | -- |
| NSB | 0.001 | -- | -- | -- |
| S1 | 2.776 | 2.775 | 500 | 14.4 |
| S2 | 0.885 | 0.884 | 250 | 7.20 |
| S3 | 0.253 | 0.252 | 125 | 3.60 |
| S4 | 0.072 | 0.071 | 62.5 | 1.80 |
| S5 | 0.024 | 0.023 | 31.3 | 0.901 |
| S6 | 0.010 | 0.009 | 15.6 | 0.450 |
| S0 | 0.004 | 0.003 | 0 | 0 |

* To convert pg/mL to µIU/mL divide by 34.7

## TYPICAL STANDARD CURVE

A typical Insulin standard curve is shown below. This curve must not be used to calculate insulin concentrations; each user must run a fresh standard curve for each assay.




## PERFORMANCE CHARACTERISTICS

### Specificity

The specificity of the assay was determined by running serial dilutions of the analyte and cross-reactants, in the assay, fitting the resulting dose response curve(s) to a 4PL curve-fit and determining the ED50. The ED50 of the standard curve was then divided by the determined ED50 of the cross-reactant and multiplied by 100.

| Analyte | Cross Reactivity |
|---|---|
| Proinsulin | < 0.8% |
| Rat insulin in serum or plasma | Not detected |
| Mouse insulin in serum or plasma | Not detected |

**Sensitivity**

The sensitivity or limit of detection of the assay is 15.5 pg/mL and was determined by interpolation at 2 standard deviations above the background (0 pg/mL) of 18 zero standard replicates.

**Intra-assay precision** was determined by assaying 20 replicates of three matrix controls containing insulin in a single assay.

| Intra-assay precision | |
|---|---|
| **pg/mL** | **%CV** |
| 257.8 | 6.3 |
| 99.1 | 6.2 |
| 50.9 | 20.2 |

**Inter-assay precision** was determined by measuring matrix controls of varying insulin concentrations in 15 assays over several days.

| Inter-assay precision | |
|---|---|
| **pg/mL** | **%CV** |
| 250 | 8.5 |
| 90.9 | 9.4 |
| 41.9 | 20.5 |

## REFERENCES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4. Cheetham, SW, Brand AH. Insulin Finds Its Niche. Science. 2013. May 17; 817-818.

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