



## **Insulin (Synthetic) ELISA**

For the quantitative measurement of synthetic insulin in serum and plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	80-SINHU-E01
Size:	96 wells
Version:	v1.2: April 28, 2010

### **ALPCO Diagnostics**

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### ***INTENDED USE***

The ALPCO Insulin (Synthetic) ELISA is designed for the quantitative determination of synthetic insulin in serum or plasma.

### ***PRINCIPLE OF THE ASSAY***

The ALPCO Insulin (Synthetic) ELISA is a sandwich type immunoassay. The 96 well microplate is coated with a monoclonal antibody specific for insulin. The Standards, Mammalian Insulin Controls and samples are added to the microplate wells with a horseradish peroxidase enzyme labeled monoclonal antibody. The microplate is then incubated on an orbital microplate shaker (700-900 rpm). After the first incubation is complete, the wells are washed with Wash Buffer and blotted dry. TMB Substrate is added and the microplate is again incubated on an orbital microplate shaker (700-900 rpm). Once the second incubation period is complete, Stop Solution is added and the optical density (OD) is measured by spectrophotometer at 450 nm with a reference wavelength of 620 nm. The intensity of the color generated is directly proportional to the amount of insulin in the sample.

### ***MATERIALS SUPPLIED***

#### **Single Plate Kit (80-SINHU-E01)**

<b>Components</b>	<b>Content</b>	<b>Quantity</b>	<b>Preparation</b>
Insulin Microplate	1 microplate	12 x 8 strip wells	Ready to use
Zero Standard (0 ng/ml)	1 vial	5 ml	Ready to use
Standards (A - E) (6, 10, 30, 75, 150 $\mu$ IU/ml)	5 vials	1 ml/vial	Ready to use
Mammalian Insulin Controls	2 vials	0.6 ml/vial	Lyophilized
Conjugate Stock	1 vial	0.9 ml	11X
Conjugate Buffer	1 bottle	9 ml	Ready to use
Wash Buffer Concentrate	1 bottle	40 ml	21X
TMB Substrate	1 bottle	12 ml	Ready to use
Stop Solution	1 bottle	12 ml	Ready to use
Plate Sealers	3	-	Ready to use

### ***MATERIALS REQUIRED BUT NOT SUPPLIED***

- Precision pipettes with disposable tips capable of dispensing 25, 75, and 100  $\mu$ l
- Repeating or multi-channel pipette
- Volumetric container
- Volumetric pipettes
- Distilled (deionized) water
- Microplate washer or wash bottle
- Orbital microplate shaker capable of 700-900 rpm
- Microplate reader with 450 and 620-650 nm filter

## ***PRECAUTIONS***

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency virus), HBV (Hepatitis B virus), and HCV (Human Hepatitis C virus). Test methods for these viruses do not guarantee the absence of virus; therefore all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. All materials derived from animal sources are BSE negative. However, all materials should be kept from ruminating animals.
3. Avoid direct contact with skin.
4. This product is not for internal use.
5. Avoid eating, drinking, or smoking when using this product.
6. Do not pipette any components by mouth.
7. Components from this kit should not be mixed with components of different lot numbers.
8. Do not use components beyond the expiration date.
9. Variations to the test procedure are not recommended and may influence the test results.

## ***STORAGE CONDITIONS***

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label. The controls are stable for 7 days at 2-8°C after reconstitution. If desired, the controls can be stored in aliquots at  $\leq -20^{\circ}\text{C}$  until needed. The controls should not be repeatedly frozen and thawed. Diluted Wash Buffer is stable for 30 days at room temperature (18-25°C).

## ***SPECIMEN HANDLING***

Serum or plasma (Heparin or EDTA) specimens are appropriate for use in this assay. No dilution or treatment of the sample is required. If a sample is  $> 150 \mu\text{IU/ml}$ , the sample should be diluted in Zero Standard and the analysis should be repeated.

Specimens can be stored at 2-8°C for 24 hours prior to analysis. Storage at  $\leq -20^{\circ}\text{C}$  for longer periods is recommended. Avoid repeated freezing/thawing of the sample.

## ***REAGENT PREPARATION***

All reagents must reach room temperature prior to preparation and subsequent use in the assay.

*Conjugate Stock (11X)* is diluted with 10 parts Conjugate Buffer. For example, to prepare enough Working Strength Conjugate for one complete plate, dilute 0.8 ml of Conjugate Stock with 8 ml of Conjugate Buffer.

*Mammalian Insulin Controls (Low and High)* are provided in a lyophilized form. Dilute each control with 0.6 ml of distilled water. Close the vial with the rubber stopper and cap, then gently swirl the vial and allow it to stand for 30 minutes prior to use. The contents should be in solution with no visible particulates. The reconstituted controls are stable for 7 days stored at 2-8°C. For longer term storage the controls should be stored in aliquots at  $\leq -20^{\circ}\text{C}$  for up to 6 months (repeated freeze/thaw cycles should be avoided). The concentration range of the controls is provided on the Certificate of Analysis enclosed with each kit; however, it is recommended that each laboratory establishes its own acceptable range.

*Wash Buffer Concentrate (21X)* is diluted with 20 parts distilled water. For example, to prepare Working Strength Wash Buffer, dilute 20 ml of Wash Buffer Concentrate with 400 ml of distilled water. Diluted Wash Buffer is stable for 30 days at room temperature (18-25°C).

### **QUALITY CONTROL**

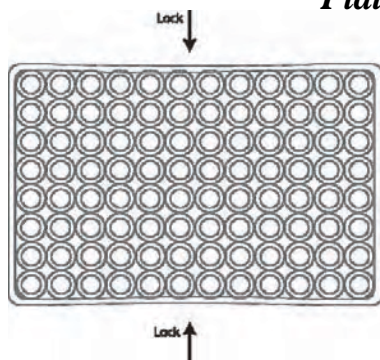
It is recommended that the controls provided with the ALPCO Insulin (Synthetic) ELISA be included in every assay. The concentration range of the controls is provided on the Certificate of Analysis enclosed with each kit; however, it is recommended that each laboratory establishes its own acceptable range.

### **ASSAY PROCEDURE**

**Bring all reagents to room temperature prior to use.** Briefly vortex all reagents before use. A standard curve must be included with each assay and/or with each plate, if more than one plate is run at a time. All standards, controls, and samples should be run in duplicate.

1. Designate enough microplate strips for the standards, controls, and desired number of samples. The remaining strips should be stored in the tightly sealed foil pouch containing the desiccant and stored at 2-8°C.
2. **Pipette 25 µl** of each standard, reconstituted control (see *Reagent Preparation*), or sample into their respective wells.
3. **Pipette 75 µl** of Working Strength Conjugate (see *Reagent Preparation*) to each well.
4. **Incubate for 1 hour**, shaking at 700-900 rpm on an orbital microplate shaker at room temperature (18-25°C).
5. **Wash the microplate 6 times** with Working Strength Wash Buffer (see *Reagent Preparation*) with a microplate washer. Alternatively, use a wash bottle to fill the wells and discard the liquid, tapping the plate on absorbent paper between washes. After the final wash, remove any residual Wash Buffer and bubbles from the wells by tapping the plate on absorbent paper towels. (See Plate Locking Diagram below.)
6. **Pipette 100 µl** of TMB Substrate to each well.
7. **Incubate for 15 minutes** on an orbital microplate shaker (700-900 rpm) at room temperature (18-25°C).
8. **Pipette 100 µl** of Stop Solution to each well. Gently mix the wells to stop the reaction. Remove bubbles before reading with microplate reader. The intensity of the yellow color is directly proportional to the concentration of insulin in the wells.
9. Place the microplate in a microplate reader capable of reading the absorbance at 450 nm with a reference wavelength of 620-650 nm. The microplate should be analyzed within 30 minutes following the addition of Stop Solution.

### **Plate Locking Diagram**



When tapping the microtiter plate onto the absorbent paper, grasp at lock points indicated by the arrows, locking the microwell strips into place.

This locking feature allows for tapping with greater force than traditional microtiter plates.

## ***CALCULATION OF RESULTS***

A calibration curve is constructed from the Standards. It is preferable to use a software program to calculate the standard curve and to determine the concentration of the samples. The preferred calculation method is cubic spline. The Zero Standard should be used as a blank with its average value subtracted from each well. Plot the standard curve using a log/log scale.

If a manual calculation is to be utilized, the Zero Standard should be used as a blank with its average value subtracted from each well. The standard concentrations are plotted on the X-axis and the absorbance values are plotted on the Y-axis. The concentrations of the unknowns are determined by plotting the absorbance of the unknown against the standard curve. The corresponding value on the X-axis is the concentration of the unknown sample.

## ***PERFORMANCE CHARACTERISTICS***

### **Sensitivity:**

The analytical sensitivity was determined by calculating the mean  $\pm 2$  standard deviations for 20 replicates of the Zero Standard. The sensitivity of the assay is 4.75  $\mu\text{IU/ml}$ .

### **Precision: Within run (intra-assay) variation**

The within run precision is expressed as the percentage coefficient of variation (%CV). This was determined based on the mean and standard deviation of 20 replicates of a specimen run in a single assay. The table below shows the results of 3 samples that span the range of the assay.

	<b>Sample 1</b>	<b>Sample 2</b>	<b>Sample 3</b>
mean	10.18 $\mu\text{IU/ml}$	18.85 $\mu\text{IU/ml}$	87.1 $\mu\text{IU/ml}$
std. dev.	0.37 $\mu\text{IU/ml}$	1.59 $\mu\text{IU/ml}$	1.87 $\mu\text{IU/ml}$
%CV	3.61 %	8.42 %	2.15 %
n=	20	20	20

### **Precision: Between run (inter-assay) variation**

The between run precision is expressed as the percentage coefficient of variation (%CV). This was determined based on the mean and standard deviation across 20 assays of duplicate measurements of a single specimen. The table below shows the results of 3 samples that span the range of the assay.

	<b>Sample 1</b>	<b>Sample 2</b>	<b>Sample 3</b>
mean	11.67 $\mu\text{IU/ml}$	19.68 $\mu\text{IU/ml}$	39.18 $\mu\text{IU/ml}$
std. dev.	1.188 $\mu\text{IU/ml}$	1.46 $\mu\text{IU/ml}$	3.78 $\mu\text{IU/ml}$
%CV	10.18 %	7.42 %	9.65 %
n=	20	20	20

**Linearity:**

The linearity of the assay was determined by preparing dilutions of a sample with a high concentration of insulin with the Zero Standard. The expected values were compared to the obtained values to determine a percent recovery. The average range of recovery was 118 -126 %.

**Spike and Recovery:**

The spike and recovery for the assay was determined by adding various known amounts of insulin to a sample. This spiked sample was evaluated in the assay and the measured concentration was compared to the expected concentration (endogenous + spiked). The range of recovery was 79-122% with an average of 99.3%.

**Specificity:**

The table below indicates the analyte and the percent cross-reactivity observed in the assay.

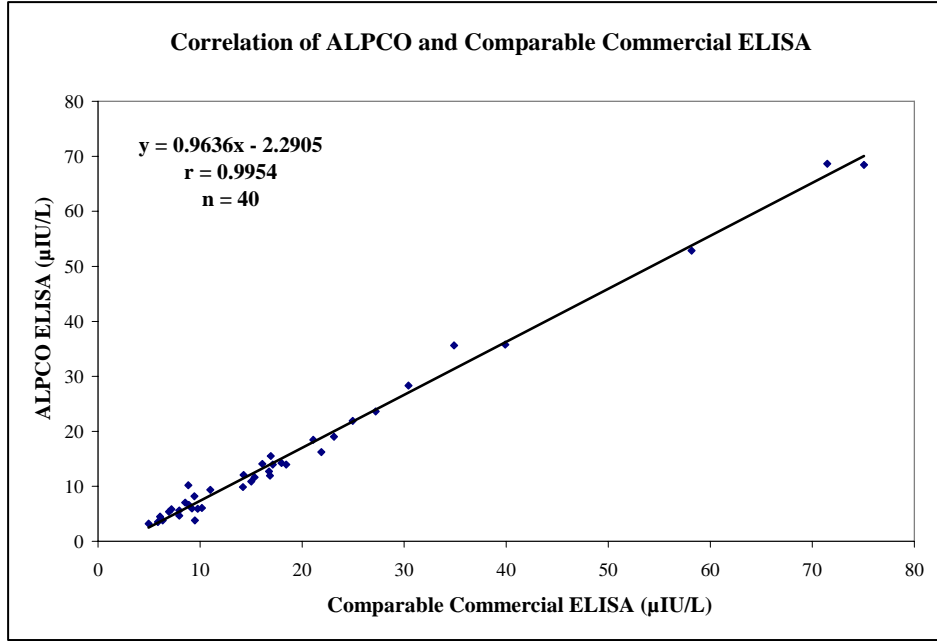
Analyte	% Cross-reactivity
Humalog	128
NovoLog	116
Humulin R	111
Humulin N	105
Lantus	46
Human insulin	88
Human C-peptide	<0.01
Human proinsulin (intact)	<0.01
Rat C-peptide 1	<0.01
Rat C-peptide 2	<0.01
Porcine insulin	81
Hamster insulin	ND
Human IGF-1	<0.01
Human IGF-2	<0.01
Mouse IGF-1	<0.01
Mouse IGF-2	<0.01

**Hook Effect:**

No high dose hook effect was observed with insulin concentrations up to 44,500  $\mu$ IU/ml.

**Accuracy:**

The ALPCO Insulin (Synthetic) ELISA was evaluated for accuracy by comparing its performance to that of another commercially available Insulin (Synthetic) ELISA. The graph below shows the results of 40 samples evaluated using both methods. The correlation coefficient between the two methods is  $r = 0.9954$ .



## Insulin (Synthetic) ELISA Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

### **80-SINHU-E01 Short Protocol:**

Insulin (Synthetic) ELISA

**Pipette 25  $\mu$ l Standards, Controls, and Samples**



**Pipette 75  $\mu$ l Conjugate**



**Incubate 1 hour (700-900 rpm) @ 18-25° C**

**Wash 6X**



**Pipette 100  $\mu$ l Substrate**



**Incubate 15 minutes (700-900 rpm) @ 18-25° C**



**Pipette 100  $\mu$ l Stop Solution, mix**



**Read @ 450 nm**