



Insulin Ultrasensitive ELISA

For the quantitative measurement of insulin in serum and plasma.

For “*In Vitro* Diagnostic” use within the United States
For Research Use Only outside of the United States

Catalog Number: 80-INSHUU-E01
Size: 1 x 96 wells

Version: v1.2: March 25, 2010

ALPCO Diagnostics

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INTENDED USE

The ALPCO Insulin Ultrasensitive ELISA is designed for the quantitative determination of insulin in human serum or plasma.

PRINCIPLE OF THE ASSAY

The ALPCO Insulin Ultrasensitive ELISA is a sandwich type immunoassay. The 96 well microplate is coated with a monoclonal antibody specific for insulin. The Insulin Standards, Diabetes Antigen Control and samples are added to the microplate wells simultaneously 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. 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 450nm with a reference wavelength of 620nm. The intensity of the color generated is directly proportional to the amount of insulin in the sample.

MATERIALS SUPPLIED

Single Plate Kit (80-INSHUU-E01)

Components	Content	Quantity	Preparation
Insulin Microplate	1 microplate	12 x 8 strip wells	Ready to use
Zero Standard	1 vial	5 ml	Ready to use
Standards (0.15, 1, 3, 10, 20 μ IU/ml)	5 vials	1 ml/vial	Ready to use
Diabetes Control	1 vial	0.6 ml/vial	Lyophilized
Conjugate Stock (11X)	1 vial	1.2 ml	Concentrate
Insulin Conjugate Buffer	1 bottle	12 ml	Ready to use
Wash Buffer (21X)	1 bottle	40 ml	Concentrate
TMB Substrate	1 bottle	12 ml	Ready to use
Stop Solution	1 bottle	12 ml	Ready to use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes with disposable tips capable of dispensing 25 μ 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 620-650nm 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 control is stable for 7 days at 2-8°C after reconstitution. If desired, the control can be stored in aliquots at $\leq -20^{\circ}\text{C}$ until needed. The control should not be repeatedly frozen and thawed. Working Strength Wash Buffer is stable for 30 days at room temperature (18-25°C). Working Strength Conjugate is stable for 30 days at 2-8°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 $> 20 \mu\text{IU/ml}$, 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 Insulin Conjugate Buffer. For example, to prepare enough Working Strength Conjugate for one complete plate, dilute 1 ml of Conjugate Stock (11X) with 10 ml of Insulin Conjugate Buffer. Working Strength Conjugate is stable for 30 days at 2-8°C.

Diabetes Control (Level 1) is provided in a lyophilized form. Dilute the 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 control is stable for 7 days stored at 2-8°C. For longer term storage the control should be aliquoted and stored at $\leq -20^{\circ}\text{C}$ for up to 6 months (repeated freeze/thaw cycles should be avoided). The concentration range of the control 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 (21X) is diluted with 20 parts distilled water. For example, to prepare Working Strength Wash Buffer, dilute 20 ml of Wash Buffer (21X) with 400 ml of distilled water. Working Strength Wash Buffer is stable for 30 days at room temperature (18-25°C).

QUALITY CONTROL

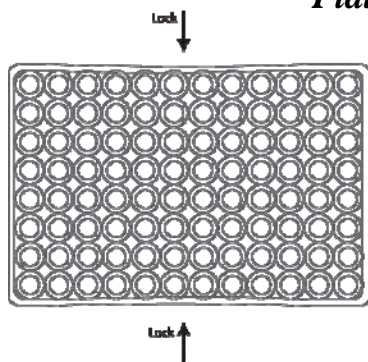
It is recommended that the control provided with the ALPCO Ultrasensitive Insulin ELISA be included in every assay. The concentration range of the control is provided on the Certificate of Analysis enclosed with each kit; however, it is recommended that each laboratory establishes its own acceptable 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, control, 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 100 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 30 minutes** at room temperature (18-25°C) on an orbital microplate shaker (700-900 rpm).
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 well.
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.

EXPECTED VALUES

The ALPCO Insulin Ultrasensitive ELISA is calibrated to the WHO insulin 1st IRP 66/304. Prior studies have determined normal insulin ranges to be 5-25 μ IU/ml. It is recommended that each laboratory establish its own normal range for its individual patient population.

Conversion for Human Insulin from International Units (IU) to grams:
1 IU of Human Insulin = 6 nmol = 34.8 μ g Insulin

PERFORMANCE CHARACTERISTICS

Sensitivity:

The analytical sensitivity was determined by calculating the mean ± 2 standard deviations for 20 replicates of the Zero Standard. The sensitivity of the assay is 0.116 μ 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 32 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.

	Sample 1	Sample 2	Sample 3
mean	6.03 μ IU/ml	12.05 μ IU/ml	16.01 μ IU/ml
std. dev.	0.13 μ IU/ml	0.48 μ IU/ml	0.39 μ IU/ml
CV%	2.22	3.98	2.5
n=	32	32	32

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 18 assays of duplicate measurements of a single specimen. The table below shows the results of 3 samples that span the range of the assay.

	Sample 1	Sample 2	Sample 3
mean	5.61 μ IU/ml	11.85 μ IU/ml	16.80 μ IU/ml
std. dev.	0.50 μ IU/ml	0.84 μ IU/ml	1.00 μ IU/ml
CV%	8.90	7.10	5.99
n=	18	18	18

Linearity:

The linearity of the assay was determined by preparing dilutions of a sample with high insulin concentration with the Zero Standard. The expected values were compared to the obtained values to determine a percent recovery. The average range of recovery was 85-95%.

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 89-128% with an average of 101%.

Specificity:

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

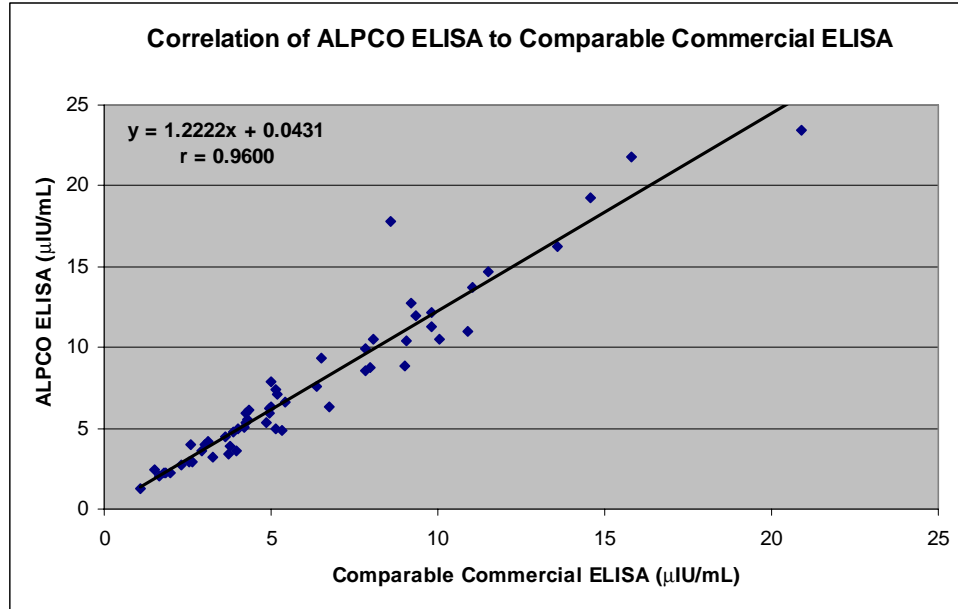
Analyte	% Cross-reactivity
Human insulin	100
Human C-peptide	ND
Human proinsulin (intact)	ND
Humalog	0.35
NovoLog	54.5
Humulin R	72.5
Humulin N	241
Lantus	42.5
Porcine insulin	175
Porcine C-peptide	ND
Human IGF-1	<0.05
Human IGF-2	<0.04

Hook Effect:

No high dose hook effect was observed with insulin concentrations up to 29,266 μ IU/ml.

Accuracy:

The ALPCO Insulin Ultrasensitive ELISA was evaluated for accuracy by comparing its performance to that of another commercially available Human Insulin Ultrasensitive ELISA. The graph below shows the results of 57 samples evaluated with both methods. The correlation coefficient between the two methods is $r = 0.960$.



Insulin Ultrasensitive ELISA Plate Map

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

80-INSHUU-E01 Short Protocol:

Insulin Ultrasensitive ELISA

Pipette 25 μ l Standards, Control, and Samples



Pipette 100 μ l Enzyme Conjugate



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

Wash 6X



Pipette 100 μ l Substrate



Incubate 30 minutes (700-900 rpm) @ 18-25° C



Pipette 100 μ l Stop Solution, mix



Read @ 450 nm