Ultrasensitive Insulin ELISA

For the quantitative determination of human insulin in serum and plasma.

For *In Vitro Diagnostic* use within the United States of America.

This product is for Research Use Only outside of the United States of America.

Catalog Number: **80-INSHUU-E01.1, E10**

Size: **96 wells, 10 x 96 wells**

Version: **May 29, 2018**

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INTENDED USE

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

PRINCIPLE OF THE ASSAY

The ALPCO Ultrasensitive Insulin ELISA is a sandwich type immunoassay. The 96-well microplate is coated with a monoclonal antibody specific for insulin. The standards, samples, and the control are added to the microplate wells with the Detection Antibody. The microplate is then incubated at room temperature on a microplate shaker at 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 incubated a second time at room temperature on a microplate shaker at 700-900 rpm. Once the second incubation is complete, Stop Solution is added, and the optical density (OD) is measured by a spectrophotometer at 450 nm. The intensity of the color generated is directly proportional to the amount of insulin in the sample.

MATERIALS SUPPLIED

80-INSHUU-E01.1		
Component	**Quantity**	**Preparation**
Insulin Microplate (96 wells)	12 x 8 strips	Ready to use
Zero Standard (0 µIU/mL)	5 mL	Ready to use
Standards (A-E) (0.15, 1, 3, 10, 20 µIU/mL)	1 mL each	Ready to use
Diabetes Control Level 1*	1 vial each	Lyophilized*
Detection Antibody	12 mL	Ready to use
Wash Buffer Concentrate	40 mL	21X
TMB Substrate	12 mL	Ready to use
Stop Solution	12 mL	Ready to use
Plate Sealers	3	Ready to use

*Please refer to the Certificate of Analysis enclosed with each kit for lot-specific control ranges and reconstitution volumes.

80-INSHUU-E10		
Component	**Quantity**	**Preparation**
Insulin Microplate (96 wells)	10 x (12 x 8 strips)	Ready to use
Zero Standard (0 µIU/mL)	5 mL	Ready to use
Standards (A-E) (0.15, 1, 3, 10, 20 µIU/mL)	1 mL each	Ready to use
Diabetes Control Level 1*	1 vial each	Lyophilized*
Detection Antibody	105 mL	Ready to use
Wash Buffer Concentrate	2 x 200 mL	21X
TMB Substrate	120 mL	Ready to use
Stop Solution	120 mL	Ready to use
Plate Sealers	20	Ready to use

*Please refer to the Certificate of Analysis enclosed with each kit for lot-specific control ranges and reconstitution volumes.

MATERIALS REQUIRED

- Precision pipettes for dispensing up to 100 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 100 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled or deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 700-900 rpm
- Microplate reader with 450 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 (Hepatitis C virus). Test methods for these viruses do not guarantee the absence of a 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 treated as potentially infectious.
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 reagents by mouth.
7. Reagents from this kit are lot-specific and must not be substituted.
8. Do not use reagents 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.

SAMPLE HANDLING

Serum and plasma (heparin or EDTA) samples are appropriate for use in this assay. No dilution or treatment of the sample is required. However, if a sample has a greater concentration of insulin than the highest standard, the sample should be diluted in Zero Standard and the analysis should be repeated.

It is recommended to 1) thoroughly vortex each sample before use and 2) perform pipetting actions without pausing.

Samples can be stored at 2-8°C for 24 hours prior to analysis. Storage at < -20°C for longer periods is recommended. Avoid repeated freeze/thaw cycles.

REAGENT PREPARATION

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

Wash Buffer Concentrate is to be diluted with 20 parts distilled water. For example, to prepare Working Strength Wash Buffer, dilute 20 mL of Wash Buffer Concentrate (21X) with 400 mL of deionized water. Working Strength Wash Buffer is stable for 4 weeks at room temperature (18-25°C).

Diabetes Control (Level 1) is provided in a lyophilized form. Please refer to the Certificate of Analysis provided with each kit for the appropriate volume of deionized water for reconstitution. Close the vial with the rubber stopper and cap, gently swirl the vial, and allow it to stand for 30 minutes prior to use. The contents of the vial should be in solution with no visible particulates. The reconstituted control is stable for 1 day stored at 2-8°C. If desired, the control can be stored at ≤ -20°C in aliquots for up to 6 months. The control should not be repeatedly frozen and thawed.

QUALITY CONTROL

It is recommended that the Diabetes 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 provided with each kit; however, it is recommended that each laboratory establishes its own acceptable range.

ASSAY PROCEDURE

All reagents and microplate strips should be equilibrated to room temperature (18-25°C) prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, samples, and the control should be run in duplicate.

1. The microplate should be equilibrated to room temperature prior to opening the foil pouch. Designate enough microplate strips for duplicate determinations of the standards, controls, and samples. The remaining microplate strips should be stored at 2-8°C in the tightly sealed foil pouch containing the desiccant.
2. **Pipette 25 µL** of each standard, control, and sample into their respective wells. See *Reagent Preparation* and Certificate of Analysis for control reconstitution instructions.
3. **Pipette 100 µL** of Detection Antibody into each well.
4. Cover microplate with a plate sealer and **incubate for 1 hour** at room temperature, shaking at 700-900 rpm on a microplate shaker.
5. Decant the contents of the wells and **wash the microplate 6 times** with 350 µL of Working Strength Wash Buffer per well (see *Reagent Preparation*) using a microplate washer. Alternatively, fill the wells with Working Strength Wash Buffer using a wash bottle equipped with a wash nozzle. (It is not recommended to use a multichannel pipette. Wash buffer must be dispensed with adequate and equal force in order to properly wash the wells.) Between washes, invert the microplate to discard the liquid and firmly tap the inverted microplate on absorbent paper towels. After the final wash, (automated or manual), remove any residual Wash Buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels.
6. **Pipette 100 µL** of TMB Substrate into each well.
7. Cover microplate with a plate sealer and **incubate for 30 minutes** at room temperature, shaking at 700-900 rpm on a microplate shaker.
8. **Pipette 100 µL** of Stop Solution into each well and gently shake the microplate to mix the contents. Remove any bubbles before proceeding with the next step.
9. Place the microplate in a microplate reader capable of reading the absorbance at 450 nm. The microplate should be analyzed immediately after the addition of the Stop Solution, and no longer than 30 minutes after.

CALCULATION OF RESULTS

Construct a standard curve from the standards. The Zero Standard should be used as a blank with its average value subtracted from each well. It is recommended to use a software program to calculate the standard curve and to determine the concentration of the samples.

The ALPCO Ultrasensitive Insulin ELISA is a ligand binding assay, with responses exhibiting a sigmoidal relationship to the analyte concentration. Currently accepted reference models for such curves use a 4 or 5-parameter logistic (pl) fit, as these models optimize the accuracy and precision across a greater range. Although cubic spline and other models are acceptable methods, they generally show less intra-assay accuracy and precision at the low and high ends of the range.

In the example below, a 5-pl curve fit was used to maximize the accuracy and precision of samples with low concentrations. However, the accuracy and precision of all models are limited

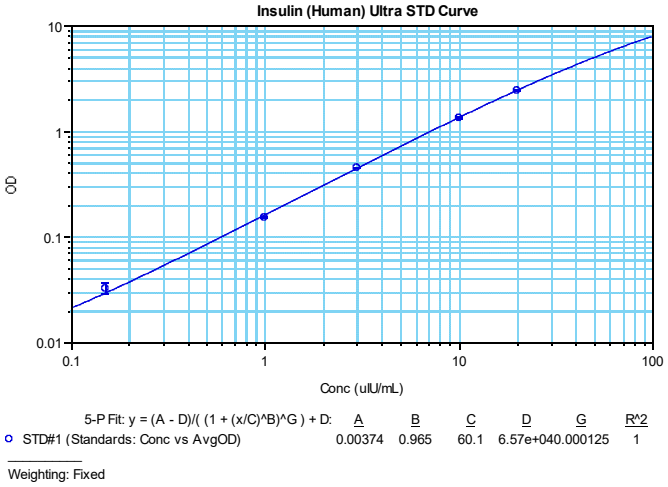
at the lowest and highest ends of the detectable range due to the influence of individual laboratory conditions. As a result, caution should always be used when interpreting results where the analyte response becomes non-linear.[1]

TYPICAL STANDARD CURVE

The following results are provided for demonstration purposes only and cannot be used in place of data obtained with the assay. A standard curve must be performed with each assay run and plate tested.

Standard	Conc. (µIU/mL)	OD
Zero	0	0
A	0.15	0.033
B	1	0.153
C	3	0.454
D	10	1.346
E	20	2.452

Blank OD	0.078



EXPECTED VALUES

The ALPCO Ultrasensitive Insulin ELISA is calibrated to the WHO Insulin First International Reference Preparation (IRP) 66/304. Prior studies have determined normal insulin ranges to be 5 – 25 µIU/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 µg 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 0.135 µIU/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 22 replicates of a sample 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	1.55 µIU/mL	4.66 µIU/mL	10.42 µIU/mL
Std. Dev.	0.17 µIU/mL	0.24 µIU/mL	0.58 µIU/mL
CV %	11.1	5.2	5.5
n	22	22	22

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 sample. The table below shows the results of 3 samples that span the range of the assay.

	Sample 1	Sample 2	Sample 3
Mean	1.63 µIU/mL	4.72 µIU/mL	9.78 µIU/mL
Std. Dev.	0.13 µIU/mL	0.23 µIU/mL	0.50 µIU/mL
CV %	8.2	4.9	5.1
n	20	20	20

Linearity
The linearity of the assay was determined by preparing dilutions of a sample with high insulin concentrations with the Zero Standard. The expected values were compared to the obtained values to determine a percent recovery. The average recovery was 93-117 %.

Spike and Recovery
The spike and recovery of 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 87 – 100 %.

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

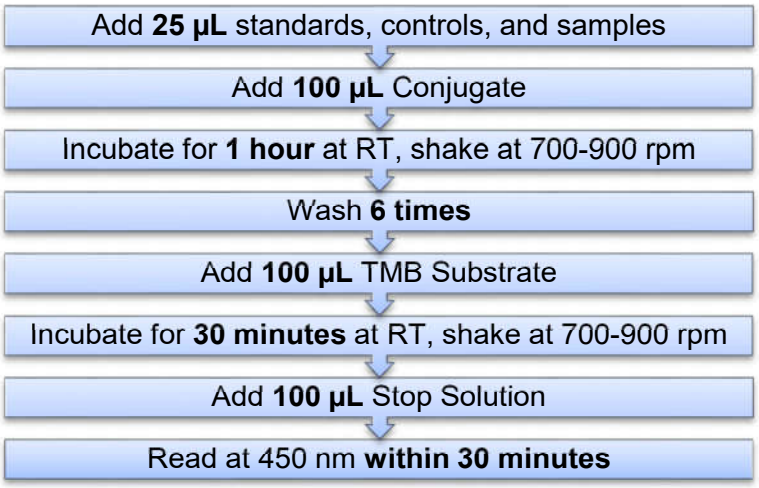
Analyte	% Cross-reactivity
Human insulin	100
Human C-peptide	Not detected
Human proinsulin (intact)	Not detected
Humalog	0.35
NovoLog	54.5
Humulin R	72.5
Humulin N	241
Lantus	42.5
Porcine insulin	175
Porcine C-peptide	Not detected
Human IGF-1	<0.05
Human IGF-2	<0.04
Mouse Insulin	Not detected
Rat Insulin	Not detected

Hook Effect
No high dose hook effect was observed with analyte concentrations up to 39,611 µIU/mL.

REFERENCES

1. Finlay JWA, Dillard RF. Appropriate Calibration Curve Fitting in Ligand Binding Assays. *AAPS Journal*. 2007; 9(2): E260-E267.

SHORT ASSAY PROTOCOL



Total Time = 1 hour, 30 minutes