



Insulin (Mouse) Ultrasensitive ELISA

For the quantitative measurement of insulin in mouse serum and plasma

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

Catalog Number: 80-INSMSU-E01
Size: 1 x 96 Wells

Catalog Number: 80-INSMSU-E10
Size: 10 x 96 Wells

Version: v1.4: February 17, 2010

ALPCO Diagnostics

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INTENDED USE

The ALPCO Insulin (Mouse) Ultrasensitive ELISA is designed for the quantitative determination of insulin in mouse serum and plasma.

PRINCIPLE OF THE ASSAY

This kit is designed for use with either 5 μ l or 25 μ l sample size. The five standards appropriate for the assay will be determined by the sample size utilized.

The ALPCO Insulin (Mouse) Ultrasensitive ELISA is a two site enzyme immunoassay. Mouse monoclonal antibody specific for insulin are immobilized to the 96-well microplate as the solid phase. Standards, controls, and samples are added to the appropriate wells with a horseradish peroxidase enzyme labeled monoclonal antibody (Conjugate), resulting in insulin molecules being sandwiched between the solid phase and the Conjugate. After incubation on a microplate shaker at room temperature, the microplate wells are washed with Wash Buffer to remove unbound Conjugate. TMB Substrate is added to each well, and the microplate is again incubated on a microplate shaker at room temperature. During the second incubation, a blue color results from TMB Substrate reacting with bound Conjugate in the wells. Stop Solution is added and this stops the reaction and changes the color from blue to yellow. The optical density (OD) is measured by microplate reader 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-INSMSU-E01)

Components	Content	Quantity	Preparation
Insulin Micoplate (coated with Mouse monoclonal anti-insulin)	1 microplate	12 x 8 well strips	Ready to use
Zero Standard (0 ng/ml)	1 vial	5 ml	Ready to use
Insulin Standards (A \rightarrow G) (0.025, 0.063, 0.188, 0.5, 1.25, 3.75, 6.9 ng/ml)	7 vials	1 ml/vial	Ready to use
Mammalian Insulin High and Low Controls	2 vials	0.6 ml/vial	Lyophilized
Conjugate (11X HRP Labeled monoclonal anti-insulin antibody)	1 vial	0.9 ml	Concentrate
Conjugate Buffer	1 bottle	9 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
Plate Sealers	3	-	Ready to use

Ten Plate Jumbo Kit (80-INSMSU-E10)

Components	Content	Quantity	Preparation
Insulin Microplate (coated with Mouse Monoclonal anti-insulin)	10 microplates	12 x 8 well strips	Ready to use
Zero Standard (0 ng/ml)	1 vial	5 ml	Ready to use
Insulin Standards (A → E) (0.025, 0.063, 0.188, 0.5, 1.25, 3.75, 6.9 ng/ml)	7 vials	1 ml/vial	Ready to use
Mammalian Insulin High and Low Controls	2 vials	0.6 ml/vial	Lyophilized
Conjugate (11X HRP Labeled monoclonal anti-insulin antibody)	1 bottle	9 ml	Concentrate
Conjugate Buffer	1 bottle	90 ml	Ready to use
Wash Buffer (21X)	2 bottles	200 ml	Concentrate
TMB Substrate	1 bottle	120 ml	Ready to use
Stop Solution	1 bottle	120 ml	Ready to use
Plate Sealers	20	-	Ready to use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes with disposable tips capable of dispensing 5 µl, 25 µl, 75 µl, and 100 µl
- Repeating or multi-channel pipette capable of dispensing 75 µl and 100 µl
- Volumetric container and pipettes for reagent preparation
- Distilled (deionized) water
- Microplate washer or wash bottle
- Horizontal 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.

SPECIMEN HANDLING

Serum and plasma specimens are appropriate for use in this assay. No dilution or treatment of the sample is required. If a sample has an insulin concentration greater than the highest standard, 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 in this assay. For longer periods, storage at $\leq -20^{\circ}\text{C}$ is recommended. Avoid repeated freezing/thawing of the sample. Grossly lipemic, icteric, or hemolyzed samples do not interfere in the assay.

REAGENT PREPARATION AND STORAGE CONDITIONS

- The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label.
- All reagents must reach room temperature prior to preparation and subsequent use in the assay.

Conjugate (11X) is diluted with 10 parts Conjugate Buffer. For example, to prepare enough Working Strength Conjugate for one complete microplate, dilute 0.9 ml of Conjugate (11X) with 9 ml of Conjugate Buffer. Working Strength Conjugate is stable for 4 weeks at 2-8°C.

Mammalian Insulin Controls (Low and High) are provided in a lyophilized form. Reconstitute 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 of the vial should be in solution with no visible particulates. The reconstituted controls are stable for 7 days stored at 2-8°C. If desired, the controls can be aliquoted and stored at $\leq -20^{\circ}\text{C}$ for up to six months. The controls should not be repeatedly frozen and thawed.

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 4 weeks at room temperature (18-25°C).

QUALITY CONTROL

It is recommended that the Mammalian Insulin High and Low Controls provided with the ALPCO Insulin (Mouse) Ultrasensitive ELISA be included in every assay. The concentration ranges of the controls are provided on the Certificate of Analysis enclosed with each kit; however, it is recommended that each laboratory establishes its own acceptable ranges.

ASSAY PROCEDURE

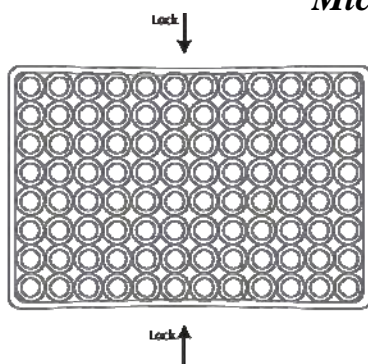
Bring all reagents and microplate strips to room temperature prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay and with each microplate if more than one is run at a time. All standards, controls, and samples should be run in duplicate.

1. Ensure that microplates are at room temperature prior to opening foil pouch. Designate enough microplate strips for the standards, controls, and desired number of samples. The remaining microplate strips should be stored at 2-8°C in the tightly sealed foil pouch containing the desiccant.
2. **Pipette 5 μl or 25 μl** of each standard, reconstituted control (see *Reagent Preparation*) or sample into its respective wells.

<p>5 μl Sample - Use standards 0.188, 0.5, 1.25, 3.75, 6.9 ng/ml (C \rightarrow G) 25 μl Sample - Use standards 0.025, 0.063, 0.188, 0.5, and 1.25 ng/ml (A \rightarrow E)</p>
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3. **Pipette 75 μ l** of Working Strength Conjugate (see *Reagent Preparation*) into each well.
4. **Incubate for 2 hours**, shaking at 700-900 rpm on a horizontal 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 then discard the liquid, inverting and firmly tapping the microplate on absorbent paper towels between washes. After the final wash with either the microplate washer or wash bottle, remove any residual Wash Buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels (See *Microplate Locking Diagram* below).
6. **Pipette 100 μ l** of TMB Substrate into each well.
7. **Incubate for 30 minutes** at room temperature (18-25°C) on a horizontal microplate shaker (700-900 rpm).
8. **Pipette 100 μ l** of Stop Solution into each well. Gently shake the microplate to stop the reaction. Remove bubbles before reading with microplate reader.
9. Place the microplate in a microplate reader capable of reading the absorbance at 450nm with a reference wavelength of 620-650nm. The microplate should be analyzed within 30 minutes following the addition of Stop Solution.

Microplate Locking Diagram



When tapping the microplate on the absorbent paper towels, grasp at lock points indicated by the arrows, locking the microplate strips into place. This locking feature allows for tapping with greater force than with traditional microplates.

CALCULATION OF RESULTS

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

Manual Calculation: 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 using log/log paper. The sample concentrations are determined by plotting the absorbance of each unknown sample 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 ± 2 standard deviations for 20 replicates of the Zero Standard. The sensitivity of the assay is 0.115 ng/ml (5 μ l sample) and 0.019 ng/ml (25 μ l sample).

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 which is run in one assay. The table below shows the results of 3 samples that span the range of the assay.

5 uL sample size	Sample 1	Sample 2	Sample 3
mean	0.44 ng/ml	1.41 ng/ml	4.24 ng/ml
std. dev.	0.041	0.064	0.209
%CV	9.30%	4.53%	4.93%
n=	20	20	20

25 uL sample size	Sample 1	Sample 2	Sample 3
mean	0.21 ng/ml	0.41 ng/ml	0.54 ng/ml
std. dev.	0.014	0.013	0.021
%CV	6.60%	3.10%	3.90%
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 10 assays of duplicate measurements of a single specimen. The table below shows the results of 3 samples that span the range of the assay.

5 uL sample size	Sample 1	Sample 2	Sample 3
mean	1.085 ng/ml	1.892 ng/ml	5.166
std. dev.	0.119	0.218	0.484
%CV	10.97%	11.49%	9.37%
n=	10	10	10

25 uL sample size	Sample 1	Sample 2	Sample 3
mean	0.214 ng/ml	0.416 ng/ml	0.558 ng/ml
std. dev.	0.012	0.023	0.032
%CV	5.83%	5.55%	5.7%
n=	10	10	10

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 recovery was 105 % (5µl sample) and 82% (25 µl sample).

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 87 -116 % with an average of 100 % (5 µl sample) and 79 -106 % with an average of 96 % (25 µl sample).

Specificity:

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

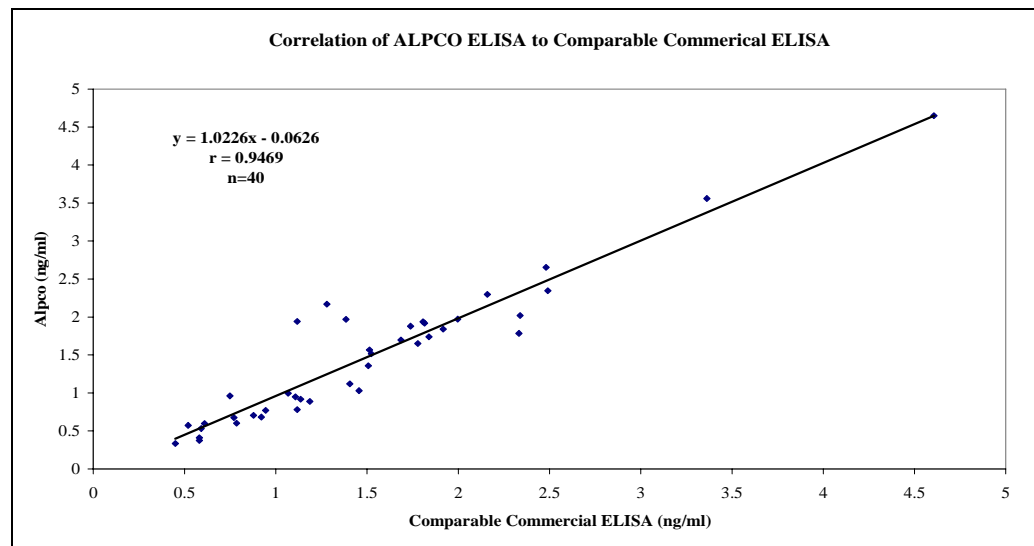
Analyte	% Cross-reactivity
Human insulin	147.0
Human C-peptide	<0.01
Human proinsulin (intact)	0.27
Humalog	153
NovoLog	180
Humulin R	253
Humulin N	305
Lantus	109
Porcine insulin	147
Porcine C-peptide	ND
Mouse C-peptide 1	<0.01
Mouse C-peptide 2	<0.01
Rat C-peptide 1	<0.01
Rat C-peptide 2	<0.01
Human 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 2662 ng/ml (5 µl sample) and up to 2048 ng/ml (25µl sample).

Accuracy:

The ALPCO Insulin (Mouse) Ultrasensitive ELISA was evaluated for accuracy by comparing its performance to that of another commercially available Mouse Ultrasensitive Insulin ELISA. The correlation coefficient between the two methods is r = 0.946 (5 µl sample).



Insulin (Mouse) Ultrasensitive ELISA Plate Map: 5 µl Sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ng/ml	0 ng/ml										
B	0.188 ng/ml	0.188 ng/ml										
C	0.5 ng/ml	0.5 ng/ml										
D	1.25 ng/ml	1.25 ng/ml										
E	3.75 ng/ml	3.75 ng/ml										
F	6.9 ng/ml	6.9 ng/ml										
G	Low Control	Low Control										
H	High Control	High Control										

80-INSMSU-E01 Short Protocol: 5 µl Sample

Insulin (Mouse) Ultrasensitive ELISA

5 µl Sample - Use standards 0.188, 0.5, 1.25, 3.75, 6.9 ng/ml

Pipette 5 µl Standards, Controls, and Samples



Pipette 75 µl Conjugate



Incubate 2 hours (700-900 rpm) @ 18-25° C

Wash X 6



Pipette 100 µl TMB Substrate



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



Pipette 100 µl Stop Solution, mix



Read @ 450 nm

Insulin (Mouse) Ultrasensitive ELISA Plate Map: 25 µl Sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ng/ml	0 ng/ml										
B	0.025 ng/ml	0.025 ng/ml										
C	0.063 ng/ml	0.063 ng/ml										
D	0.188 ng/ml	0.188 ng/ml										
E	0.5 ng/ml	0.5 ng/ml										
F	1.25 ng/ml	1.25 ng/ml										
G	Low Control	Low Control										
H												

80-INSMSU-E01 Short Protocol: 25 µl Sample

Insulin (Mouse) Ultrasensitive ELISA

25 µl Sample - Use standards 0.025, 0.063, 0.188, 0.5, and 1.25 ng/ml

Pipette 25 µl Standards, Controls, and Samples



Pipette 75 µl Conjugate



Incubate 2 hours (700-900 rpm) @ 18-25° C

Wash X 6



Pipette 100 µl TMB Substrate



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



Pipette 100 µl Stop Solution, mix



Read @ 450 nm