



Insulin (Rat) Ultrasensitive ELISA

For the quantitative measurement of insulin in rat serum and plasma.

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

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

Catalog Number: 80-INSRTU-E10
Size: 10 x 96 wells

Version: v1.3: March 9, 2010

ALPCO Diagnostics

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INTENDED USE

The ALPCO Insulin (Rat) Ultrasensitive ELISA is designed for the quantitative determination of insulin in rat serum or 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 (Rat) Ultrasensitive 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 at 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 at 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-INSRTU-E01)

Components	Content	Quantity	Preparation
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 - G) (0.02, 0.05, 0.15, 0.4, 1, 3, 5.5 ng/ml)	7 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

Ten Plate Jumbo Kit (80-INSRTU-E10)

Components	Content	Quantity	Preparation
Insulin Microplate	10 microplates	12 x 8 strip wells	Ready to use
Zero Standard (0 ng/ml)	1 vial	5 ml	Ready to use
Standards (A-G) (0.02, 0.05, 0.15, 0.4, 1, 3, 5.5 ng/ml)	7 vials	1 ml/vial	Ready to use
Mammalian Insulin Controls	2 vials	0.6 ml/vial	Lyophilized
Conjugate Stock	1 bottle	9 ml	11X
Conjugate Buffer	1 bottle	90 ml	Ready to use
Wash Buffer Concentrate	2 bottles	200 ml	21X
TMB Substrate	1 bottle	120 ml	Ready to use
Stop Solution	1 bottle	120 ml	Ready to use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes with disposable tips capable of dispensing 5 μ l and 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 620-650 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 aliquoted and stored at $\leq -20^{\circ}\text{C}$ until needed. The controls should not be repeatedly frozen and thawed. Working Strength Wash Buffer is stable for 30 days at room temperature (18-25°C).

SAMPLE HANDLING

Serum or plasma samples are appropriate for use in this assay. No dilution or treatment of the sample is required. If a sample is greater than the highest standard, the sample should be diluted in Zero Standard and the analysis should be repeated.

Samples 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 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 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. Working Strength 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 (Rat) 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 to room temperature prior to use. Briefly vortex all reagents before use. A standard curve must be run with each assay and/or with each plate, if more than one plate is used during a day. All standards, controls, and samples should be run in duplicate.

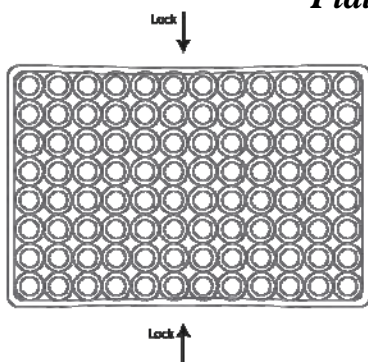
1. Designate enough microplate strips for the standards, controls, and desired number of samples. The remaining strips should be stored in the tightly sealed foil pouch containing the desiccant and stored at 2-8°C.
2. **Pipette 5 μl or 25 μl** of each standard, reconstituted control (see *Reagent Preparation*),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

5 μl Sample - Use standards 0.15, 0.4, 1.0, 3.0, and 5.5 ng/ml
25 μl Sample - Use standards 0.02, 0.05, 0.15, 0.4, and 1.0 ng/ml

3. **Pipette 75 μl** of Working Strength Conjugate (see *Reagent Preparation*) into each well.
4. **Incubate for 2 hours**, 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 final wash, remove any residual Wash Buffer and bubbles from the wells prior to proceeding to the next step. (See Plate 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 an orbital microplate shaker at 700-900 rpm.
8. **Pipette 100 μ l** of Stop Solution into 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.

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 0.107 ng/ml (5 μ l sample) and 0.002 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 sample run in a single assay. The table below shows the results of 3 samples that span the range of the assay.

5 µl sample	Sample 1	Sample 2	Sample 3
mean	3.165 ng/ml	1.932 ng/ml	4.071 ng/ml
std. dev.	0.267 ng/ml	0.094 ng/ml	0.132 ng/ml
CV%	8.44%	4.89%	3.24%
n=	20	20	20

25 µl sample	Sample 1	Sample 2	Sample 3
mean	0.532 ng/ml	0.941 ng/ml	0.374 ng/ml
std. dev.	0.02 ng/ml	0.039 ng/ml	0.017 ng/ml
CV%	3.73%	4.14%	4.44%
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 sample. The table below shows the results of 3 samples that span the range of the assay.

5 µl sample	Sample 1	Sample 2	Sample 3
mean	2.94 ng/ml	1.92 ng/ml	3.84 ng/ml
std. dev.	0.28 ng/ml	0.13 ng/ml	0.29 ng/ml
CV%	9.5%	6.86%	7.45%
n=	10	10	10

25 µl sample	Sample 1	Sample 2	Sample 3
mean	0.34 ng/ml	0.52 ng/ml	0.89 ng/ml
std. dev.	0.01 ng/ml	0.03 ng/ml	0.08 ng/ml
CV%	4.36%	6.29%	9.40%
n=	10	10	10

Linearity:

The linearity of the assay was determined by preparing dilutions of a sample with a high insulin concentration with the Zero Standard. The expected values were compared to the obtained values to determine a percent recovery. The range of recovery was 96 - 118 % with an average of 107 % (5 µl sample) and 76 - 136 % with an average of 109 % (25 µl assay).

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 101-119 % with an average of 110 % (5 µl sample) and 95-107 % with an average of 102 % (25 µl sample).

Specificity:

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

Analyte	% Cross-reactivity
Human insulin	120
Human C-peptide	<0.01
Human proinsulin (intact)	0.18
Humalog	115
Novolog	140
Humulin R	200
Humulin N	229
Lantus	83.8
Porcine insulin	113
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 1,659 ng/ml (5 µl sample) and up to 1,495 ng/ml (25 µl sample).

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

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

80-INSRTU-E01 Short Protocol:

Insulin (Rat) Ultrasensitive ELISA

5 µl Sample - Use standards 0.15, 0.4, 1.0, 3.0, and 5.5 ng/ml

Pipette 5 µl Standards, Controls, and Samples



Pipette 75 µl Conjugate



Incubate 2 hours (700-900 rpm) @ 18-25° C
Wash 6X



Pipette 100 µl TMB Substrate



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



Pipette 100 µl Stop Solution, mix



Read @ 450 nm

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

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

80-INSRTU-E01 Short Protocol:

Insulin (Rat) Ultrasensitive ELISA

25 µl Sample - Use standards 0.02, 0.05, 0.15, 0.4, and 1 ng/ml

Pipette 25 µl Standards, Controls, and Samples



Pipette 75 µl Conjugate



Incubate 2 hours (700-900 rpm) @ 18-25° C
Wash 6X



Pipette 100 µl TMB Substrate



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



Pipette 100 µl Stop Solution, mix



Read @ 450 nm