



Insulin (Porcine/Canine) ELISA

For the quantitative measurement of insulin in Porcine/Canine serum and plasma (EDTA)

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

Catalog Number:	80-INSPO-E01
Size:	96 wells
Version:	v1.1: April 20, 2010

ALPCO Diagnostics

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INTENDED USE

The ALPCO Insulin (Porcine/Canine) ELISA is for the quantitative determination of insulin in Porcine/Canine serum and plasma.

PRINCIPLE OF THE ASSAY

The ALPCO Insulin (Porcine/Canine) ELISA is a sandwich type immunoassay. Mouse monoclonal antibodies 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 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-INSPO-E01)

Components	Content	Quantity	Preparation
Insulin Microplate (coated with mouse monoclonal anti-insulin antibody)	1 microplate	12 x 8 well strips	Ready to use
Zero Standard (0 ng/ml)	1 vial	5 ml	Ready to use
Standards (A → E) (0.05, 0.1, 0.3, 1.0, 2.0 ng/ml)	5 vials	1 ml/vial	Ready to use
Mammalian Insulin High and Low Controls	2 vials	0.6 ml/vial	Lyophilized
Conjugate Stock (HRP Labeled monoclonal anti-insulin antibody)	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 µl, 75 µl, and 100 µl
- Repeating or multi-channel pipette capable of dispensing 75 µl and 100 µl
- Volumetric containers 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 620-650 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.

SPECIMEN HANDLING

Serum and plasma (EDTA) specimens are appropriate for use in this assay. No dilution or treatment of the sample is required. If a sample contains > 2.0 ng/ml of insulin, 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 ≤ -20°C is recommended. Avoid repeated freezing/thawing of the sample. Grossly lipemic, icteric, or hemolysed 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 Stock 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 Stock (11X) with 9 ml of Conjugate Buffer. Working Strength Conjugate is stable for 30 days at 2-8°C.

Mammalian Insulin High and Low Controls are provided in a lyophilized form.

Reconstitute the controls with 0.6 ml of distilled water. Close the vials with the rubber stopper and cap, then gently swirl the vials and allow them to stand for 30 minutes prior to use. The contents of the vials 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 ≤ -20°C for up to 6 months. The controls should not be repeatedly frozen and thawed.

Wash Buffer Concentrate is 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 distilled water. Working Strength Wash Buffer is stable for 30 days at room temperature (18-25°C).

QUALITY CONTROL

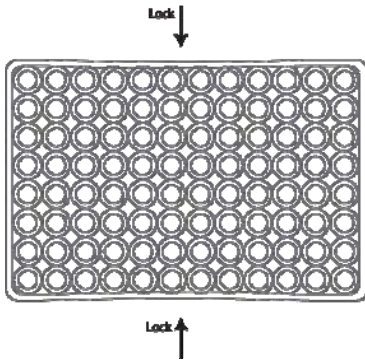
It is recommended that the Mammalian Insulin High and Low Controls provided with the ALPCO Insulin (Porcine/Canine) ELISA be included in every assay. The concentration ranges of the controls are provided on the Certificate of Analysis enclosed with each kit; however, it is recommended that each laboratory 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 in the tightly sealed foil pouch containing the desiccant at 2-8°C.
2. **Pipette 25 µl** of each standard, reconstituted control (see *Reagent Preparation*) or sample into its respective well.
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 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 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 µl** of TMB Substrate to each well.
7. **Incubate for 15 minutes** at room temperature (18-25°C) on a horizontal microplate shaker (700-900 rpm).
8. **Pipette 100 µl** of Stop Solution to each well. Gently shake the microplate to stop the reaction. Remove bubbles before reading with the microplate reader.
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.

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

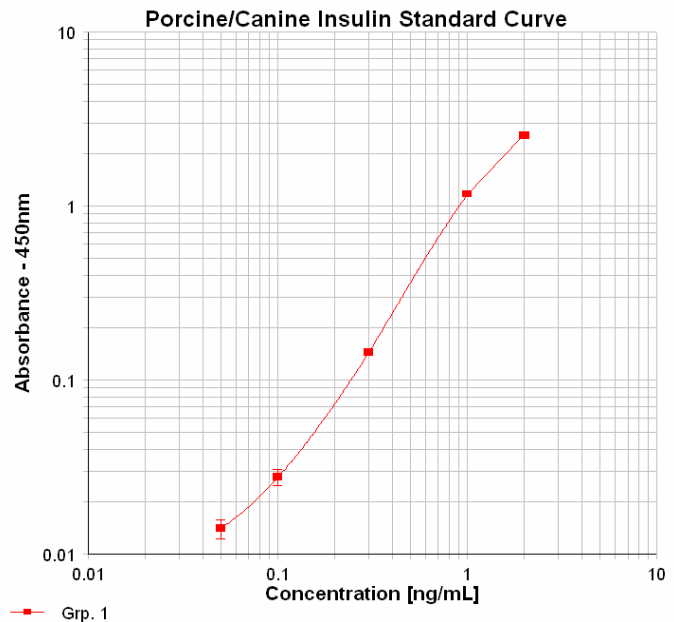
A calibration curve is constructed from the Standards. It is preferable to use a software program to calculate the standard curve and to determine the concentration of the samples. The Zero Standard should be used as a blank with its average value subtracted from each well. The preferred calculation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

TYPICAL STANDARD CURVE

The following results are provided for demonstration purposes only and cannot be used instead of data obtained from the actual assay. Each laboratory must determine the standard curve with each assay and plate tested.

Standard	Absorbance - 450 nm
0 ng/ml	0.005
0.05 ng/ml	0.014
0.1 ng/ml	0.028
0.3 ng/ml	0.144
1.0 ng/ml	1.17
2.0 ng/ml	2.53



PORCINE 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 0.007 ng/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 24 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	0.255 ng/ml	0.414 ng/ml	0.984 ng/ml
std. dev.	0.01 ng/ml	0.03 ng/ml	0.06 ng/ml
%CV	4.0%	6.7%	6.4%
n=	24	24	24

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.

	Sample 1	Sample 2	Sample 3
mean	0.207 ng/ml	0.356 ng/ml	0.937 ng/ml
std. dev.	0.017 ng/ml	0.030 ng/ml	0.082 ng/ml
%CV	8.2%	8.4%	8.7%
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 105-147% with an average of 125%.

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 84-100% with an average of 92%.

Specificity:

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

Analyte	% Cross-reactivity
Porcine insulin	100
Human insulin	38
Human C-peptide	<0.01
Human proinsulin (intact)	0.18
Humalog	100
Mouse C-peptide 1	<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 310 ng/ml.

CANINE 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 0.007 ng/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.

	Sample 1	Sample 2	Sample 3
mean	1.217 ng/ml	0.823 ng/ml	0.135 ng/ml
std. dev.	0.04 ng/ml	0.03 ng/ml	0.01 ng/ml
%CV	2.9%	4.2%	8.7%
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.

	Sample 1	Sample 2	Sample 3
mean	1.144 ng/ml	0.794 ng/ml	0.161 ng/ml
std. dev.	0.55 ng/ml	0.034 ng/ml	0.017 ng/ml
%CV	4.8%	4.3%	10.4%
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 119-127 % with an average of 122%.

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 87-124% with an average of 109%.

Specificity:

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

Analyte	% Cross-reactivity
Porcine insulin	100
Human insulin	38
Human C-peptide	<0.01
Human proinsulin (intact)	0.18
Humalog	100
Mouse C-peptide 1	<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 310 ng/ml.

Insulin (Porcine/Canine) ELISA Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ng/ml	0 ng/ml										
B	0.05 ng/ml	0.05 ng/ml										
C	0.1 ng/ml	0.1 ng/ml										
D	0.3 ng/ml	0.3 ng/ml										
E	1.0 ng/ml	1.0 ng/ml										
F	2.0 ng/ml	2.0 ng/ml										
G	Low Control	Low Control										
H	High Control	High Control										

