ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
N-CAL™ KIT

NCal™ International Standard Kit

DetectX®

Insulin
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K046-H1

Species Independent

Sample Types Validated:

Serum, Plasma and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

WEB 180816

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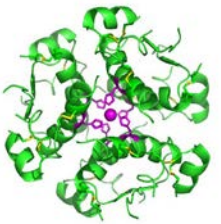
BACKGROUND

The human insulin protein is a 51 amino acid anabolic peptide-hormone that is secreted by the pancreatic β-cells in the Islets of Langerhans. Insulin consists of two chains (A and B) connected by disulfide bonds[1]. One of its primary functions is the stimulation of glucose uptake from the systemic circulation, as well as the suppression of hepatic gluconeogenesis, thereby serving a major role in glucose homeostasis and preventing the metabolic disorder *diabetes mellitus*. The work of Banting, Best, Collip and MacCleod in the early 1920's resulted in the identification of a substance in extracts of pancreas that had the remarkable ability to reduce blood glucose levels in diabetic animals[2] and by 1923 these pancreas extracts were being used to successfully treat diabetic patients. Insulin exists primarily as a monomer at low concentrations (~10^{-6} M) and forms dimers at higher concentrations at neutral pH[3]. At high concentrations and in the presence of zinc ions insulin aggregates further to form hexameric complexes[4]. Preproinsulin, the first translational product from the insulin gene, is a 110 amino acid polypeptide with a 24 amino acid signal peptide.



Insulin Hexamer

The major function of insulin is to counter the concerted actions of a number of hyperglycemia-generating hormones and to maintain low blood glucose levels. In addition to its role in regulating glucose metabolism, insulin stimulates lipogenesis, diminishes lipolysis, and increases amino acid transport into cells. Because there are numerous hyperglycemic hormones, untreated disorders associated with insulin generally lead to severe hyperglycemia and shortened life span. Insulin also exerts activities typically associated with growth factors. Insulin is a member of a family of structurally and functionally similar molecules that include the insulin-like growth factors and relaxin. The tertiary structure of all four molecules is similar, and all have growth-promoting activities. Insulin modulates transcription and stimulates protein translocation, cell growth, DNA synthesis, and cell replication; effects that it holds in common with the insulin-like growth factors and relaxin[5, 6].

1. Sanger, F., "The Chemistry of Insulin," Science, 1959, 129:1340-1344.
2. Bliss, M., "The Discover of Insulin," University of Chicago Press, Chicago, 1982.
3. Frank, BH., Pekar, AH., & Veros, AJ., "Insulin and proinsulin conformation in solution," Diabetes [Suppl. 2], 1972, 21:486-4914.
4. Blundell, T, Dodson, G, Hodgkin, D, Mercola, D., "Insulin: the structure in the crystal and its reflection in chemistry and biology.", Adv. Protein Chem., 1972, 26:279-402.
5. Nishi M, and Nanjo K., "Insulin gene mutations and diabetes.", J. Diab. Invest., 2011, 2:92-99.
6. Taniguchi, CM, Emanuelli, B and Kahn CR, "Critical nodes in signalling pathways: insights into insulin action," Mol. Cell. Biol., 2006, 7:85-96.

ASSAY PRINCIPLE

The DetectX® Insulin EIA kit is designed to quantitatively measure insulin present in a variety samples and tissue culture media. Please read the complete kit insert before performing this assay. An Insulin standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture insulin present in the sample. After a 60 minute incubation, the plate is washed. A peroxidase conjugated insulin monoclonal antibody is added and the plate is again incubated for 30 minutes and washed. Substrate is then added to the plate, which reacts with the bound insulin conjugated antibody. After a third incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the Insulin in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Corticosterone EIA & CLIA Kits	K014-H1/H5, K014-C1/C5
Cortisol EIA Kits	K003-H1/H1W/H5/H5W
Cyclic AMP Direct EIA & CLIA Kits	K019-H1/H5, K019-C1/C5
Galactose Colorimetric Detection Kit	K042-H1
Glucose Colorimetric Detection Kit	K039-H1
Glucose Fluorescent Detection Kit	K039-F1
Hemoglobin High Sensitivity Colorimetric Detection Kit	K013-HX1/HX5
Prostaglandin E_2 (PGE_2) Multi-Format Kits	K051-H1/H5
Testosterone EIA Kits	K032-H1/H5
Thyroxine (T_4) EIA Kits	K050-H1/H5
Triiodothyronine (T_3) EIA Kit	K056-H1/H5

SUPPLIED COMPONENTS

Clear Coated 96 Well Plate
Clear plastic microplate with break-apart strips coated with monoclonal antibody to human Insulin.

One Plate — Catalog Number C187-1EA

Human Insulin Standard
Insulin at 64,000 pg/mL in a special stabilizing solution.

40 µL — Catalog Number C188-40UL

DetectX® Insulin Conjugate
A monoclonal antibody to human Insulin labeled with peroxidase.

5 mL — Catalog Number C186-5ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.

28 mL — Catalog Number X065-28ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.

30 mL — Catalog Number X007-30ML

TMB Substrate
11 mL — Catalog Number X019-11ML

Stop Solution
A 1M hydrochloric acid solution. **CAUSTIC**.

5 mL — Catalog Number X020-5ML

Plate Sealer
2 each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

Once opened the kit can be stored at 4°C up to the expiration date on the kit label.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes.

Repeater pipet and disposable tips capable of dispensing 100 µL and 50 µL.

A microplate washer.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for human serum, plasma, and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using. Due to the highly conserved nature of insulin it is expected that this kit will measure human, bovine and porcine insulin (See page 14 for bovine and porcine reactivity). This assay has low or no reactivity to rat or mouse insulin. The end user should test this kit for application in their samples.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples must be diluted ≥ 1:5 in Assay Buffer.

Tissue Culture Media Samples

TCM samples should be diluted ≥ 1:4 in Assay Buffer and read off the standard curve generated in Assay Buffer. RPMI-1640, diluted 1:4 in Assay Buffer, was validated in this kit.

Any samples with concentrations outside the standard curve range should be diluted further with Assay Buffer, as appropriate, to obtain readings within the standard curve range.

Use all samples within 2 hours of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

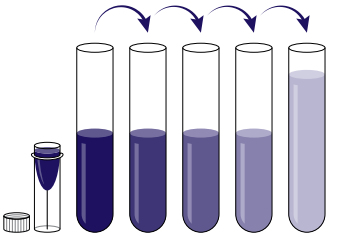
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Standard Preparation

Label test tubes as #1 through #5. Pipet 390 µL of Assay Buffer into tubes #1. Pipet 200 µL of Assay Buffer into tubes #2 to #5. Carefully add 10 µL of the 64,000 pg/mL Insulin standard to tube #1 and vortex completely. Take 200 µL of the Insulin solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #5. The concentration of Insulin in the tubes #1 through #5 will be 1,600, 800, 400, 200 and 100 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5
Assay Buffer Volume (µL)	**390**	200	200	200	200
Addition	**Stock**	Std 1	Std 2	Std 3	Std 4
Volume of Addition (µL)	**10**	200	200	200	200
Final Conc (pg/mL)	**1,600**	800	400	200	100

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Insulin concentrations.

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of Assay Buffer into the zero standard wells. Cover the plate with the plate sealer and shake at room temperature for 60 minutes. *NOTE: Non-shaking reduces signal by ~ 30%.*
4. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
5. Add 50 µL of the DetectX® Insulin Conjugate to each well, using a repeater pipet.
6. Cover the plate with the plate sealer and shake at room temperature for 30 minutes.
7. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
8. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
9. Incubate the plate at room temperature for 30 minutes, without shaking.
10. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
11. Read the optical density generated from each well at 450 nm.
12. Use the plate reader's built-in 4PLC software capabilities to calculate Insulin concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-insulin-eia-kit.assay

MyAssays

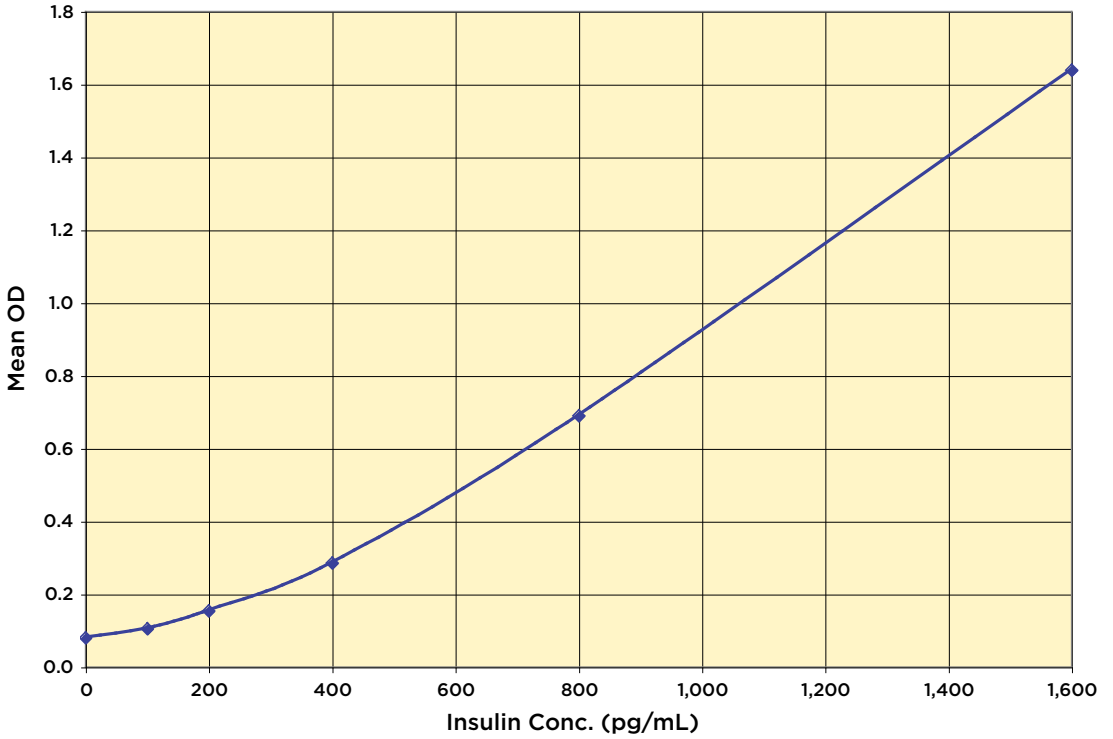
TYPICAL DATA

Sample	Mean OD	Insulin Conc. (pg/mL)
Standard 1	1.639	1,600
Standard 2	0.690	800
Standard 3	0.286	400
Standard 4	0.154	200
Standard 5	0.106	100
Zero	0.080	0
Sample 1	0.484	609.1
Sample 2	0.233	327.7

Always run your own standard curve for calculation of results. Do not use this data.

43.3 pg/mL human Insulin is equal to 1 µIU/mL. 1 ng of human Insulin is equivalent to 0.023 milliunits of the WHO 1st International Standard 1975 (66/304)

Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

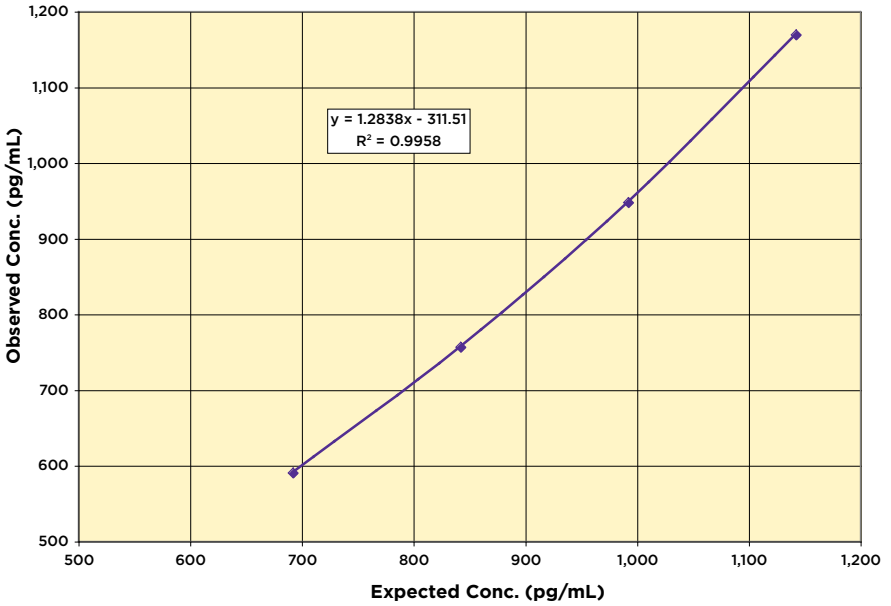
Sensitivity was calculated by comparing the OD's for twenty-two wells run for each of the zero and standard #5. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 48.8 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration serum sample. **Limit of Detection was determined as 27.8 pg/mL.**

Linearity

Linearity was determined by taking two diluted serum samples, one with a low diluted Insulin level of 542.0 pg/mL and one with a higher diluted level of 1,292.5 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the values previously determined.

High Sample	Low sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	1142.4	1169.3	102.4%
60%	40%	992.3	947.7	95.5%
40%	60%	842.2	756.4	89.8%
20%	80%	692.1	590.7	85.3%
			Mean Recovery	**93.3%**



Intra Assay Precision

Three serum samples were diluted with Assay Buffer and run in replicates of ≥ 18 in an assay. The mean and precision of the calculated Insulin concentrations were:

Sample	Insulin Conc. (pg/mL)	%CV
1	1,013.5	1.5
2	665.3	3.2
3	371.8	2.4

Inter Assay Precision

Three serum samples and three commercial control sera were diluted with Assay Buffer and run in duplicates in sixteen assays run over multiple days by three operators. The mean and precision of the calculated Insulin concentrations were:

Sample	Insulin Conc. (pg/mL)	%CV
1	655.7	8.9
2	348.7	10.6
3	382.6	11.6
4	340.8	8.8
5	637.6	6.6
6	1,039.5	5.3

SAMPLE VALUES

A number of human serum samples were tested in the kit. Normal human serum levels ranged from 511.8 to 4,340 pg/mL with an average of 1,930.3 pg/mL. Serum from a Humalog® treated type 1 diabetic ranged from 18,161 pg/mL to 65,036 pg/mL with an average of 39,288 pg/mL. Samples were diluted 1:5 up to 1:40 fold in Assay Buffer.

Humalog® is recombinant human insulin manufactured by Eli Lilly and Company.

CROSS REACTIVITY

The following cross reactants were tested in the assay and cross reactivity calculated within the standard curve.

Steroid	Cross Reactivity (%)
human Insulin	100%
porcine Insulin	301.9%
bovine Insulin	267.7%
human glucagon	0.03%
human C peptide	0.03%
human proinsulin	< 0.01%
rat Insulin	< 0.01%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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