ELISA kits available from ADI (see details at the web site)

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin
#1200 Human Albumin (Urinary)
#1750 Human IgG (total) **#1760** Human IgM
#1800 Human IgE **#1810** Human Ferritin
#1210 Human Transferrin (Tf) **#0020** Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125)
#1830 Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA)
#1510 free PSA (fPSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin
#0040 Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG)
#0410 HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4)
#1110 Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3)
#1700 Human T3 (total)

#1850 Human Cortisol **#1860** Human Progesterone
#1865 Human Pregnolone **#1875** Human Aldosterone
#1880 Human Testosterone **#1885** Human free Testosterone
#1910 Human Androstenedione **#1920** Human Estradiol
#1925 Human Estrone **#1940** Dihydrotestosterone (DHT)
#1950 Human DHEA-sulphate (DHEA-S)
#3400 Human serum Neopterin

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA
#3200 Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-0350

Glucagon Like Peptide (GLP-1) Active

ELISA KIT Cat. No. 0350

For Quantitative Determination of Active GLP-1 (GLP-1, 7-36 amide and GLP-1 (7-37) In Plasma of Human, Mouse, and Rat.

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Web Site: www.4adi.com

Human GLP-1 Active ELISA KIT # 0350,
Kit Contents: (reagents for 96 tests)

Components	Cat#
Anti-GLP-1 precoated microwell **strip plate** (96 wells). Ready-to-use	0351
GLP-1 (7-36) amide, **Standard**, 2 vials, lyophilized, dissolve in **0.5** ml water for stock conc. of 160 pmol/L	0352
Anti-Antibody (GLP-1 IgG)-**HRP Conj (30X). 0.4 ml**	0353
HRP **Dilution buffer, 12 ml**	0354
GLP-1 **Assay buffer. 30 ml**	0355
Wash Buffer (40X), 50 ml	WB-350
TMB substrate Solution, **15 ml**	TMB-350
Stop solution (diluted sulfuric acid), **12 ml**	ST-350
Complete Instruction Manual	M-0350

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is a 31 amino acid peptide hormone derived from selective cleavage of the proglucagon gene. It is mainly produced from enteroendocrine L-cells in GI tract. The other cleavage products derived from proglucagon genes are glucagon, GLP-2 and other small fragment peptides including Glicentin, Oxyntomodulin and two intervening peptides (IP-1 and IP-2). Except for glucagon cleaved in α cells of the pancreas, all other cleaved peptides occurred in enteroendocrine L cells of intestine.

Incretins are a group of gastrointestinal hormones that cause an increase in the amount of insulin released from the beta cells of the islets of Langerhans after eating. They also inhibit glucagon release from the alpha cells of the islets of Langerhans. As a result, they slow the rate of absorption of nutrients into the blood stream by reducing gastric emptying and may directly reduce food intake. The two main candidate molecules that fulfill criteria for an incretin are glucagon-like peptide-1 (GLP-1) and Gastric inhibitory peptide (or glucose-dependent insulinotropic polypeptide or GIP). The human proglucagon gene was cloned in 1983, and the human proglucagon sequence was subsequently deduced. After that, it was found that the specific sequence of GLP-1 has insulinotropic effect: GLP-1 (7-36) amide. Now, GLP-1 (7-36) amide and GLP-1 (7-37) are known as active forms of GLP-1. They are rapidly inactivated to GLP-1 (9-36) amide and GLP-1 (9-37) by DPP-IV within a few moments in blood. Currently, Several DPP-IV inhibitors that can be taken orally as a tablet have already been developed as a treatment for diabetes.

This ELISA kit can measure **Active forms of GLP-1 (GLP-1 (7-36) amide and GLP-1 (7-37)** specifically in human plasma. Due to conservation of GLP-1, this kit can also be used for mouse, rat samples. Note: **We recommend to use DPP-IV inhibitor when collecting blood to prevent degradation of GLP-1.**

ADI GLP-1 ELISA kit is intended for *in vitro research* use only (RUO).

Species Reactivity

GLP-1 antibody reacts with the mouse, rat, and human GLP-1 so this ELISA test is recommended for mouse and rat samples as well.

QUALITY CONTROL

Additional controls should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. When the laboratory test the GLP-1 assay, the release of patient sample results should be based on whether the kit Control results fall within the suggested acceptable ranges. If one or more of the quality control sample values lie outside the acceptable limits, the assay should be repeated. Once the laboratory has generated data of its own, the quality control parameters should be based on the statistical data by the laboratory, using either kit Control and/or serum pools made by the laboratory. Levy-Jenning plots on control results should be used. If the results for all the control samples are within mean + 2 standard deviations, with no definitive trend or bias of the quality control data, the assay should be deemed acceptable. The Westgard rule should be followed to be compliant with CLIA 88 regulations. If the control results do not fall within the stated parameters as described, assay results are invalid.

LIMITATIONS OF THE PROCEDURE

Serum samples or other fluids have not been tested, we recommend testing or other samples with appropriate controls.

References

Orskov C 91994) Diabetes 43, 535-539; Yanihara N (1990) Hum. Cell. 3, 1-8; Asarian L (1998) Physiol. Behav. 64, 367-372; Mojsov S (1990) JBC 265, 8001-8008; Vella A (2000) Diabetes 49, 611-617; Nauck MA (1998) Daibetes Med. 15, 937-945; Naslun E (2004) Br. J. Nutr. 91, 439-446;

Related ELISA kits

Catalog#	ProdDescription
0030-10-B1	Bovine Insulin ELISA Kit, 96 tests, Quantitative, 96 tests, Quantitative
0030-20-I	Human Insulin-Biotin ELISA Kit, 96 tests, Quantitative, 96 tests
0030-40-1	Mouse Insulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-40-10	Mouse Insulin ELISA Kit, High Sensitivity, Quantitative, 10x 96 tests
0030-50-1	Rat Insulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-50-10	Rat Insulin ELISA Kit, High Sensitivity, Quantitative, 10x 96 tests
0030-60-1	Mouse/Rat Proinsulin ELISA Kit, High Sensitivity, Quantitative, 96
0030-70-1	Mouse/Rat C-Peptide ELISA Kit, High Sensitivity, Quantitative, 96
0030N	Human Insulin ELISA Kit, 96 tests, Quantitative, 96 tests
0035-IA	Human Insulin & Insulin Analogs (Lispro/Humalog, Aspart, Glargine, Glulisine, Determir) ELISA Kit, 96 tests,
0040	Human C-peptide ELISA Kit, 96 tests, Quantitative

Preparation of 7-working GLP-1 Standards

Take 1 vial of GLP-1 (7-36 amide) and dissolve in 0.5 ml of distilled water. Mix it gently to prepare 160 pmol/L initial stock. This will be diluted 1:2 fold to prepare 7 standards of 80-1.25 pmol/L as follows.

Std#	Stock GLP-1	GLP-1 Assay buffer	Total Volume*	Final Conc pmol/L
1.	250 ul of **160 pmol/L**	250 ul	500 ul	80
2.	250 ul of **80 pmol/L**	250 ul	500 ul	40
3.	250 ul of **40 pmol/L**	250 ul	500 ul	20
4.	250 ul of **20 pmol/L**	250 ul	500 ul	10
5.	250 ul of **10 pmol/L**	250 ul	500 ul	5
6.	250 ul of **5 pmol/L**	250 ul	500 ul	2.5
7.	250 ul of **2.5 pmol/L**	250 ul	500 ul	1.25
8.	blanks	250ul	-	0

*=500 ul will become 250 ul because 250 ul will be transferred to make the next standards. You need 200 ul of each stds to test (100 ul/well x 2). Prepare the standards. It is preferable to make the stds on the day of the test and do not store for more than 1-2 days.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE 22-28 oC BEFORE USE).*

Arrange required # of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Pipet **100 ul** of blanks, standards (1-8), controls and samples into appropriate wells in *duplicate*. **Mix gently and incubate for 60 min at 37oC.**

2. First Aspirate the well contents completely and **wash the wells 6-7 times with 1x wash buffer (300 ul/well)** We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add **100 ul** of 1X **Antibody-HRP conj** into each well. Mix gently for 5-10 seconds and **incubate for 60 min at 2-4oC.**

4. Aspirate the well contents completely and **wash the wells 6-7 times with 1x wash buffer (300 ul/well)** as in step 2. After the last wash, plate must be tapped over paper towel to remove traces of HRP.

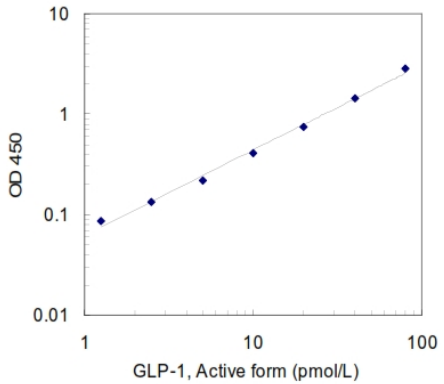
5. Dispense **100 ul of TMB substrate** into the wells. Mix gently for 5-10 seconds, cover the plate and **incubate for 30 min at room temp (22-28oC) in the drawer or covered with foil.** Positive wells will develop blue color.

6. Add **100 ul stop solution** into each well and mix gently for 5-10 seconds (blue color turns into yellow). **Read absorbance at 450 nm** and 630 as reference within 15 minutes.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (pmol/L)	Net Abs.	Calculated Conc (pmol/l)
A1, A2	**Std. A (blanks)**	0.058	
B1, B2	**Std. B** 1.25	0.144	
C1, C2	**Std. C, 2.5**	0.193	
D1, D2	**Std. D**, 5	0.275	
E1, E2	**Std. E** , 10	0.469	
E1, E2	**Std. F** , 20	0.809	
E1, E2	**Std. G** , 40	1.499	
E1, E2	**Std. G** , 80	2.889	
G1, G2	**Sample 1**	0.801	20

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values.



CALCULATION OF RESULTS

1. Deduct the A450 values (blanks) from standards and all samples.

2. Assign the concentration for each calibrator stated on the vial in mU/ml. Plot the data from the calibration curve on semil-log graph paper with the concentration on the X-axis and the corresponding A450 values on the Y-axis.

3. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation. Obtain the concentration of the sample by locating the absorbance unit on the Y-axis and finding the corresponding concentration value on the X-axis. Patient and control samples should be read using the standards curve.

4. if using graphic software programs, we recommend using cubic spline or 4 PL [4 Parameter Logistics] or Point-to-Point can generally give a good fit.

Sensitivity

0.10 pmol/L (1 pmol=3 pg)

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

Performance Characteristics

1. Added Recovery Assay

Specimen	Theoretical Value (pmol/L)	Measurement Value (pmol/L)	%
*Human Plasma (EDTA) (x2)	41.40	33.93	82.0
	21.40	18.91	88.4
	11.40	9.96	87.4
*Mouse Plasma (EDTA) (BALB/c) (x2)	42.51	39.93	93.9
	22.51	20.93	93.0
	12.51	11.54	92.2
*Rat Plasma (EDTA) (SD) (x2)	21.24	17.75	83.6
	11.24	9.20	81.9
	6.24	5.19	83.2
10%FCS added RPMI-1640 (x2)	40.41	38.02	94.1
	20.41	20.33	99.6
	10.41	9.96	95.7

*the plasma samples was contained in DPP-IV inhibitors.

2. Intra-Assay

Measurement Value (pmol/L)	SD value	CV value (%)	n
41.56	2.26	5.4	24
11.38	0.51	4.5	24
2.43	0.13	5.5	24

2. Inter-Assay

Measurement Value (pmol/L)	SD value	CV value (%)	n
41.62	3.03	7.36	7
11.24	0.8	7.1	7
2.18	0.24	10.9	7

4. Specificity

Compound	Crossreactivity
GLP-1 (7-36)amide	100%
GLP-1 (7-37)	100%
GLP-1 (1-37)	0.32%
GLP-1 (9-36) amide, GLP-2, Glucagon, Human/mouse GIP	<0.1%

PRINCIPLE OF THE TEST

GLP-1 ELISA kit is based on sequential binding of GLP-1 from samples to two antibodies, one immobilized on microtiter well plates and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of GLP-1 present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Control and Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

The determination of GLP-1 should be performed on human EDTA plasma samples. **We recommend using DPP IV inhibitors or BD collection tubes that contains DPPIV. The DPPIV prevent GLP-1 degradation in the sample. It is better to test the samples as soon as possible. Sample dilutions, if required, should be performed in ELISA buffer.**

Avoid repeated freezing and thawing of samples. For long term storage of samples, it is recommended that samples should be aliquoted into sample tubes or vials prior to freezing. Prior to use, allow all specimens to come to room temperature (22oC to 28oC) and mix by gentle inversion or swirling. Avoid grossly hemolyzed or grossly lipemic samples.

Reagent Preparation

1. **Prepare 1X wash buffer from 40X stock.** Dilute 50 mL of **40X Wash buffer Conc**. with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

2. **Prepare 1X Antibody-HRP Conjugate from 30X stock.** Prepare 900 ul for every 8-well strip or 10 ml for full plate. Example, 30 ul stock conjugate and 870 ul of Anti-HRP dilution buffer or 0.33 ml stock conjugate in 0.966 mls for a full plate assay. Note: Do not store diluted conjugate beyond the assay date and prepare in required volumes only.

3. Preparation of Working standards. (see detailed procedure on page 3).