

Glicentin ELISA

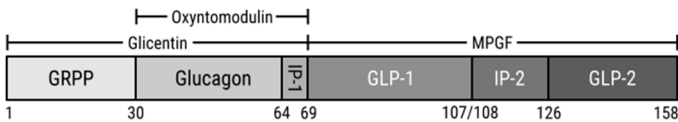
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AL-185

INTENDED USE

The Glicentin enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Glicentin in EDTA plasma and other biological fluids. This kit is intended for research use only.

SUMMARY AND EXPLANATION



Glicentin is a 69 amino acid N-terminal peptide derived from proglucagon (PG 1-69) by posttranslational processing in the intestine by intestinal L-cells. Glicentin can further be processed into oxyntomodulin, corresponding to proglucagon residues no 33-69. These peptides are released simultaneously upon stimulation. Glicentin is a pro-glucagon derived gut peptide that is released in the gut in response to feed intake. It is present in high levels in the circulation after stimulation and is often a problem when measuring glucagon because of its shared sequence.

PRINCIPLE OF THE TEST

The Glicentin ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Glicentin antibody coated microtiter wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated Glicentin antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of Glicentin in the samples and calibrators.

MATERIALS SUPPLIED

CAL-185A – CAL-185F Glicentin Calibrators A-F (Lyophilized)

Six vials, labeled A-F, containing concentrations of approximately 0 – 1666 pg/mL Glicentin in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators A-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze in plastic vials immediately for multiple use and discard after the run. Avoid repeated freeze thaws. The Glicentin concentration in the calibrators are traceable to the manufacturer's working calibrators. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-185-I & CTR-185-II Glicentin Controls I & II

Two vials, labeled Levels I and II containing low and high Glicentin concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and Freeze immediately in plastic vials for multiple use and discard after running. Avoid repeated freeze thaws.

PLT-185 Glicentin Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with Glicentin antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

ASB-185 Glicentin Assay Buffer

One bottle, 12 mL containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

CND-185 Glicentin Conjugate Diluent

One bottle, 12 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

BCC-185 Glicentin Biotin Conjugate Concentrate (50X)

One vial, 0.4 mL, containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Dilute prior to use in CND-172. Store at 2-8°C until expiration date.

SAR-185 Glicentin Streptavidin-Enzyme Conjugate Ready-to-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
2. Microplate shaker.
3. Microplate washer.

4. Semi-automated/manual precision pipette to deliver 10–250 μL .
5. Vortex mixer.
6. Deionized water.

WARNINGS AND PRECAUTIONS

For research use only. Not for use in diagnostic procedures.

The following precautions should be observed:

- a) Follow good laboratory practice.
- b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
- c) Handle and dispose of all reagents and material in compliance with applicable regulations

WARNING: Potential Biohazardous Material

This reagent may contain some animal source material (e.g. BSA) or materials used in conjunction with animal source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.¹

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide² as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

chlorohydrocarbons often found in laboratory water supplies. Use deionized water.

5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **Glicentin calibrators A-F and Controls I & II:** Tap and reconstitute Glicentin Calibrator A-F and Controls I & II each with **1 mL** deionized water. Solubilize, mix well and use after reconstitution. To homogenize leave for 10 min before use.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.
4. **Glicentin Antibody-Biotin Conjugate Solution:** The Glicentin Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1-part conjugate to 50 parts of CND-185, according to the number of wells used. If an entire plate is to be used, pipet exactly 220 μL of the Concentrate in to 11 mL of the diluent.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use. Calibrators, Controls, and Unknowns should be assayed in duplicate.

1. Label the microtitration strips to be used.
2. Pipette **25 μL** of the Calibrators (Cal A-F), Control and Unknowns to the appropriate wells.
3. Add **100 μL** of the Glicentin Assay Buffer to each well using a repeater pipette.
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **90 minutes** at room temperature ($23\pm 2^\circ\text{C}$).
5. Aspirate and wash each strip **5 times** with Wash Solution using an automatic microplate washer.
6. During the last **20-30 minutes** of incubation, prepare the Antibody-Biotin Conjugate Solution by diluting the Glicentin Biotin Conjugate Concentrate in Biotin Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
7. Add **100 μL** of the Glicentin Antibody Biotin Conjugate Solution to each well using a repeater pipette.
8. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature ($23\pm 2^\circ\text{C}$).
9. Aspirate and wash each strip **5 times** with Wash Solution using an automatic microplate washer.
10. Add **100 μL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
11. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature ($23\pm 2^\circ\text{C}$).
12. Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
13. Add **100 μL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
14. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **10-12 min** at room temperature ($23\pm 2^\circ\text{C}$). NOTE: Visually monitor the color development to optimize the incubation time.

SAMPLE COLLECTION

- a) K_2EDTA Plasma is the recommended sample type.
- b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.
- c) Samples may be stored at 4°C if assayed within 24 hours; otherwise samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.
- d) Avoid assaying lipemic, hemolyzed or icteric samples.
- e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Glicentin assay. It is the customer's responsibility to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic

15. Add **100 µL** of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.

NOTE: Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.

RESULTS

NOTE: The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log Glicentin concentration on X-axis** using a cubic regression curve-fit. Alternatively, linear regression curve-fit can be used. Other data reduction methods may give slightly different results.

- Calculate the mean optical density (OD) for each Calibrator, Control, or Unknown.
- Optimum results can be obtained at incubation temperature of $(23 \pm 2^\circ\text{C})$.
- Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Glicentin concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
- Determine the Glicentin concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Glicentin concentrations.
- Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (Cal. A) and re-assayed.
- Any sample reading lower than the analytical sensitivity should be reported as such.
- Multiply the value by a **dilution factor**, if applicable.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Glicentin levels in human **EDTA plasma**. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.³

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Glicentin ELISA controls or other commercial controls should fall within 100%
- The confidence limits for Glicentin controls are printed on the **Calibration Card**.
- A full calibration curve, low- and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean Absorbance	Conc. (pg/mL)
A1, A2	A	0.051 (Blank)	0
B1, B2	B	0.076	33.3
C1, C2	C	0.207	100
D1, D2	D	0.577	266
E1, E2	E	1.550	733
F1, F2	F	2.830	1666

CAUTION: The above data must not be employed in lieu of data obtained by the user in the laboratory.

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in pg/mL.

1 pg/mL = 0.125 pmol/L

Analytical Sensitivity:

The analytical sensitivity in the Glicentin ELISA assay, as calculated by the interpolation of mean plus two standard deviations of 19 replicates of calibrator A (0 pg/mL) and calibrator B (33.33 pg/mL), is **4.17 pg/mL**.

Linearity:

Two Human EDTA plasma samples and calibrator F containing various Glicentin levels were diluted with Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	%Recovery
Sample-1	Neat	410.690		
	1:2	205.089	194.488	95%
	1:4	102.544	94.908	92%
Sample-2	Neat	213.208		
	1:2	106.604	105.851	99%
	1:4	53.302	44.050	83%
Calibrator F	Neat	1666		
	1:2	833	842.219	101%
	1:4	416.500	369.203	89%
	1:8	208.250	190.492	91%
	1:16	104.125	94.650	91%

Imprecision:

Reproducibility of the Glicentin assay was determined in a study using two kit controls. The study included a total of four assays, replicates of six per assay (n=24). Representative data were calculated and are presented in the following table.

Sample	Mean Conc. (pg/mL)	Within run		Between run		Total	
		SD	%CV	SD	%CV	SD	%CV
Control I	252.58	11.64	4.61%	4.07	1.61%	12.33	4.88%
Control II	659.87	20.12	3.05%	6.40	0.97%	21.11	3.20%

Recovery

Two low reading Glicentin sample were spiked into calibrator F and assayed. The spiked recovery is shown below.

Sample ID	Endogenous Value (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	% Recovery
1	24.512	434.89	387.43	89%
2	0.0010	416.50	359.68	86%

Analytical Specificity:

The Glicentin sequence is highly conserved among mammals. The Antibody pair used in Glicentin assay detects Bovine, Porcine, Goat, Canine, Sheep and Squirrel monkey and cross-reactivity to other closely related analytes is listed below.

Cross-Reactant	Concentration	% Cross-reactivity
Oxyntomodulin	1000 ng/mL	ND
Glucagon (1-29)	1000 ng/mL	ND
GLP-1 (1-36)	1000 ng/mL	ND

GLP-1 (7-36 amide)	1000 ng/mL	ND
GLP-1 (9-36 amide)	1000 ng/mL	ND
GLP-2 (1-34)	1000 ng/mL	ND
GRPP	1000 ng/mL	ND
MPGF-I	10 ng/mL	ND
MPGF-II	10 ng/mL	ND
Insulin	10 ng/mL	ND
Thyroglobulin	10ng/mL	ND
C-peptide	10 ng/mL	ND

ND=Not Detectable

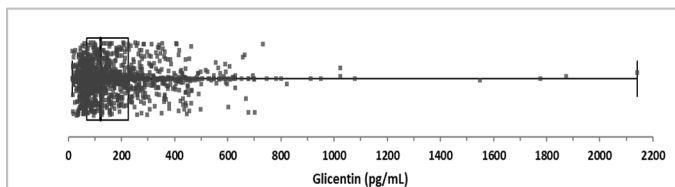
Interference:

When potential interferents (hemoglobin, biotin, intralipids and bilirubin) were added at a concentration greater than two folds of their physiological concentration to control sample, average Glicentin concentration were within $\pm 10\%$ of the control as represented in the following table.

Interferent	Interferent Dose	Sample (pg/mL)	Dosed Sample (pg/mL)	% Difference
Hemoglobin	1 mg/mL	572.4	588.9	2.9
	0.5 mg/mL	621.0	632.3	1.8
	0.1 mg/mL	622.0	636.9	2.4
Hemoglobin	1 mg/mL	309.6	325.9	5.3
	0.5 mg/mL	289.5	310.3	7.2
	0.1 mg/mL	338.2	341.8	1.1
Biotin	1200 ng/mL	581.2	587.9	1.2
	600 ng/mL	634.0	625.0	-1.4
Biotin	1200 ng/mL	265.7	285.0	7.3
	600 ng/mL	323.8	328.5	1.5
Intralipids	20 mg/mL	583.5	577.8	-1.0
	10 mg/mL	660.9	643.3	-2.7
	5 mg/mL	590.9	609.3	3.1
Intralipids	20 mg/mL	305.9	324.5	6.1
	10 mg/mL	308.9	324.8	5.2
	5 mg/mL	338.9	351.3	3.6
Bilirubin	0.66 mg/mL	449.8	449.1	-0.2
	0.2 mg/mL	598.3	587.9	-1.7
Bilirubin	0.66 mg/mL	222.5	216.8	-2.6
	0.2 mg/mL	310.3	305.2	-1.6

Expected Values:

Expected Glicentin concentration in undifferentiated population (diabetic and non-diabetic) was calculated by evaluating 1526 samples in Ansh Labs Glicentin ELISA. The frequency distribution was calculated using Analyse-It® for Microsoft Excel and is shown below.



n	Glicentin (pg/mL)			
	Mean	Median	Range	95% CI
1526	174.4	122.5	14.6 - 2141.8	29.9 - 592.3
Quantile		Glicentin (pg/mL)		
0.100		48.3		
0.200		61.8		
0.300		78.7		
0.400		97.7		
0.500		122.5		
0.600		150.5		
0.700		193.8		
0.800		257.8		
0.900		369.8		

NOTE: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

REFERENCES

- HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available <http://www.cdc.gov/biosafety/publications/bmbl5/BMML5>
- DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available: <http://www.cdc.gov/niosh>.
- Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037-1038.

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