Mercodia

Northern Lights Mercodia Total GLP-1 NL-ELISA

Directions for Use

10-1278-01
Reagents for 96 determinations

For Research Use Only
Not for Use in Diagnostic Procedures

Manufactured by

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

Explanation of symbols used on labels

	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
	Lot No.

Intended use

Mercodia Total GLP-1 NL-ELISA provides a method for the quantitative determination of amidated GLP-1 in human plasma and serum samples.

Summary and explanation of the test

Glucagon-like-peptide-1, GLP-1, is one of the major incretin hormones produced in the intestinal L-cells. It is secreted as the active forms (7-37)/(7-36) amide but is rapidly cleaved by the enzyme DPP4 forming the metabolites GLP-1 (9-37)/(9-36) amide[1]. Although the two active forms have shown equal biological potency, the majority of circulating GLP-1 in humans is found in the amidated form.[2,3] It is also the amidated forms that increase significantly after a meal, keeping the non-amidated forms at a more basal level.[2]

In order to study GLP-1 secretion in vivo it is necessary to measure the metabolite (9-36) amide since only small amounts of the intact hormone will be found in the circulation[4].

GLP-1 is released post-prandially in the intestine and has the ability to increase glucose-stimulated insulin secretion and inhibit glucagon secretion[5,6]. The dual influence that GLP-1 exerts on pancreatic function has made it an attractive target for pharmacological interventions, giving rise to the development of peptide mimetics and inhibitors of DPP4.

Principle of the procedure

Mercodia Total GLP-1 NL-ELISA is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the GLP-1 molecule. GLP-1 in the sample reacts with anti-GLP-1 antibodies bound to microtitration wells and peroxidase-conjugated anti-GLP-1 antibodies in the solution. A simple washing step removes unbound enzyme-labelled antibody. The bound conjugate is detected by reaction with the chemiluminescent substrate. A luminescence plate reader is used to read the intensity of light generated.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- Instrument settings should be optimized according to the manufacturer's instructions.
- Not for internal or external use in humans or animals.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, 5, 6 and 7 and Wash Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

The Enzyme Conjugate Buffer, the Calibrators and Wash Buffer are labeled:

Warning

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

Material required but not provided

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution and substrate working solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader for chemiluminescence
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

Reagents 1 X 96

Each Mercodia Total GLP-1 NL-ELISA kit (10-1278-01) contains reagents for 96 wells, sufficient for 40 samples and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2–8°C.

Coated Plate Mouse monoclonal anti-GLP-1 For unused microplate wells completely reseal the bag using adhesive tape, store at 2–8°C and use within 2 months.	1 plate	96 wells 8-well strips	Ready for Use
Calibrators 1, 2, 3, 4, 5, 6, 7 Synthetic GLP-1 (9-36) amide Color-coded yellow Concentration stated on vial label Reconstituted Calibrators are stable for 1 month at 2-8°C. If reconstituted Calibrators are to be used for longer than 1 month, aliquote and store at -20°C. Aliquoted Calibrators are stable for at least 2 months at -20°C. Avoid repeated freeze/thaw cycles.	7 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial
Calibrator 0 Color coded yellow	1 vial	5 mL	Ready for Use
Enzyme Conjugate 11X Mouse monoclonal anti-GLP-1	1 vial	0.6 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	6 mL	Ready for use
Wash Buffer 21X mL Storage after dilution: to 2-8°C for 2 months	1 bottle	50 mL	Dilute with 1000 redistilled water make wash buffer 1X solution
Substrate Reagent A Colorless solution Store the substrate working solution protected from light and use within 1 day	1 vial	7 mL	Mix 1:1 with Substrate Reagent B to make substrate working solution e.g. 5 mL + 5 mL
Substrate Reagent B Colorless solution	1 vial	7 mL	Preparation see Substrate Reagent A

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the blue Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently. Use within 1 week.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	0.36 mL	3.6 mL
4 strips	0.18 mL	1.8 mL

Specimen collection and handling

Serum or plasma can be used. However, GLP-1 in serum or EDTA plasma samples will be sensitive to storage conditions and freeze-thaw cycles. It is recommended to keep samples on ice when preparing the assay. Return to freezer as soon as possible.

Serum

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

EDTA plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant, and separate the plasma fraction by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

Preparation of samples

No dilution is normally required, however, samples above the obtained value of Calibrator 7 should be diluted with Calibrator 0. Note! Buffers containing sodium azide (NaN_3) cannot be used for sample dilution.

Test procedure

All reagents and samples must be brought to room temperature before use.

Prepare a calibrator curve for each assay run. The product has been optimized and validated without plate sealer.

1. Prepare enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare substrate working solution by mixing equal volumes of Substrate Reagent A and Substrate Reagent B, e.g. 5 mL + 5 mL. Store the mixture at room temperature and protect from light.
3. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
4. Pipette 25 µL each of Calibrators, controls and samples into appropriate wells.
5. Add 50 µL of enzyme conjugate 1X solution into each well.
6. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C).
7. On an automatic plate washer, use plate mode combined with overflow wash function to wash the plate with 700 µL wash buffer 1X solution per well for 6 cycles. Invert and tap the plate firmly against absorbent paper after the final wash.
   Or manually:
   Discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1X solution to each well. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times for a total of 6 cycles. See TN34-0106 for details.
8. Add 100 µL substrate working solution into each well.
9. Incubate on the bench for 15 minutes in the dark at room temperature (18-25°C).
10. Use a microplate reader for chemiluminescence. Measure all visible light (glow) with an integration time of 1 second. No filter is needed. Use settings for a 96 well plate with flat bottom. Other settings should be used according to the manufacturer's instructions. Read within 15 minutes.

    Note! Be extra careful not to contaminate the substrate working solution with enzyme conjugate solution.

Internal quality control

Internal plasma pools with low, intermediate and high GLP-1 concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution and/or reconstitution dates of kit components, relative light units (RLU) values for the blank and Calibrators and concentration values for the controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

Calculation of results

The concentration of GLP-1 is obtained by plotting the relative light units (RLU) of the calibrators, except for Calibrator 0, versus their concentration. It is important to use an appropriate curve fitting model that represent the true dose-response relationship to get accurate results. It is every laboratory's responsibility to try out the functionality of the chosen curve fitting model and used software. Note that weighting of the curve fit is important to get a proper fit at the low range of the standard curve, especially when the measuring range is wide.

The Mercodia Total GLP-1 NL-ELISA is validated with Five parameter logistic with weighting $1/y^2$, using Magellan (Tecan) software.

Example of results

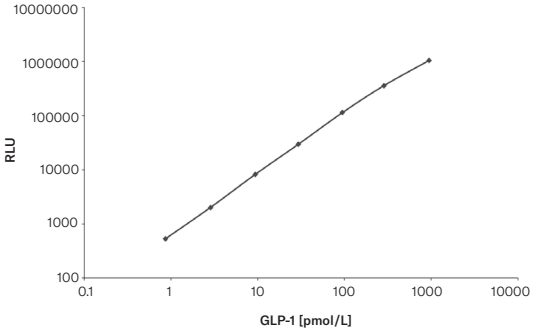
These values were obtained using Tecan Infinite M200 with 1 s integration time.

Wells	Identity	RLU	Mean Conc. pmol/L
1 A-B	Calibrator 0	125/151	
1 C-D	Calibrator 1*	521/538	
1 E-F	Calibrator 2*	2000/2015	
1 G-H	Calibrator 3*	8313/8084	
2 A-B	Calibrator 4*	29625/29608	
2 C-D	Calibrator 5*	114160/114440	
2 E-F	Calibrator 6*	359680/357720	
2 G-H	Calibrator 7*	1042300/1053200	
3 A-B	Sample 1	3090/2877	3.96
3 C-D	Sample 2	592730/602660	481
3 E-F	Sample 3	3087/3042	4.06

*Concentration stated on vial label

Example of calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



Conversion factor

1 pg/mL = 0.32 pmol/L

Limitations of the procedure

Samples from patients undergoing dulaglutide treatment may give falsely low values as dulaglutide may interfere with assay results.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.

Fasting levels for 113 tested, apparently healthy individuals, yielded a mean of 9.2 pmol/L, a median of 6.9 pmol/L and central 95% reference range of 2-25 pmol/L analyzed in P800 plasma.

Performance characteristics

The assay has been validated according to CLSI[7-12], FDA[13] and EMA[14] guidelines. Selected studies are presented here. Additional data can be obtained from Mercodia.

Validation of curve fit

The curve fitting was validated with Five parameter logistic with weighting using $1/y^2$. Cubic spline regression and Four parameter logistic with weighting $1/s^2$ will also give acceptable results.

Sensitivity and range of quantification

Analytical sensitivity (or Capability of Detection) is 0.65 pmol/L as determined by the methodology described in ISO11843-Part 4.

Functional sensitivity (or Lower Limit of Quantification, LLOQ) is 1.0 pmol/L as determined according to FDA/EMA guidelines.

The Upper Limit of Quantification, ULOQ, is 940 pmol/L as determined according to FDA/EMA guidelines.

Precision and accuracy

Each sample was analyzed in 4 replicates on at least 23 different occasions by four different technicians.

Sample	Mean value pmol/L	Coefficient of variation		
		Accuracy %	Repeatability %*	Within laboratory %**
QC_{LLOQ}	1.27	100	6.7	11
QC_{Low}	3.52	101	4.4	9.2
QC_{Medium}	460	103	3.0	5.6
QC_{High}	672	102	2.5	4.4
QC_{ULOQ}	815	87	3.2	6.3

*Within assay variation
**Total assay variation

Selectivity and specificity

Dilutional linearity

Samples were spiked above the highest calibrator concentration and subsequently diluted for analysis in the assay. Nominal values were used for calculation.

Mean recovery for dilutional linearity is 99% (93%-106%) with precision of the final concentration across all dilutions ≤ 3%.

Parallelism

Samples with high concentration of endogenous GLP-1 were diluted 1/2, 1/4 and 1/8.

Mean recovery for parallelism is 92% (82%-109%) with precision between samples in the dilution series ≤ 11%.

High dose hook effect

Samples with a concentration up to at least 18 780 pmol/L can be measured without giving falsely low results.

Cross-reactivity
The following cross-reactions were found:

	Crossreaction (%)	Concentrations tested (pmol/L)
GLP-1 (1-36) amide	88%	1.0 - 305
GLP-1 (7-36) amide	93%	0.7 - 117
GLP-1 (9-36) amide	100%	*
GLP-1 (1-37)	n.d.	16 - 1000
GLP-1 (7-37)	n.d.	3.9 - 500
Glucagon	n.d.	1.5 - 125
GIP	n.d.	4.7 - 3000
GLP-2	n.d.	7.1 - 458
Oxyntomodulin	n.d.	4.3 - 276
Glicentin	n.d.	4.7 - 300
Miniglucagon	n.d.	4.7 - 300
MPGF	n.d.	16 - 1000
Liraglutide	n.d.	125 - 8000
Exenatide	n.d.	125 - 32000
Lixisenatide	n.d.	125 - 8000
Dulaglutide	0.07%	125 - 8000

*Calibrator material
n.d. = not detected

Interference

Recovery values at LLOQ and ULOQ concentration of GLP-1 is presented below. The substance is concluded to interfere if the recovery value is not within 100 ± 25% of the nominal concentration.

	Concentration (pmol/L)	Recovery (%) LLOQ	ULOQ
Liraglutide	8	102	96
	31	96	96
	125	99	96
	500	101	96
	2000	87	96
	8000	89	96
Exenatide	8	99	97
	31	100	98
	125	91	94
	500	97	95
	2000	100	98
	8000	102	102
Lixisenatide	8	93	98
	31	113	100
	125	96	94
	500	97	96
	2000	100	96
	8000	92	99
Dulaglutide	8	87	99
	31	86	100
	125	85	95
	500	77	96
	2000	77	78
	8000	N/A*	31

*N/A = not available, level below detection limit

Selectivity

Grossly lipemic, icteric or haemolyzed samples do not interfere in the assay.

Calibration

Mercodia Total GLP-1 NL-ELISA is calibrated against a highly purified, fully validated, commercial GLP-1 (9-36) amide preparation.

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorised distributors, in such event, shall not be liable for damages indirect or consequential.

References

1. Mentlein *et al* (1993) Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-I (7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum Eur. *J. Biochem.* 214, 829-835
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3. Orskov C *et al.* (1993) . Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes* 42(5):658-661.
4. Holst JJ and Madsbad S (2016) Mechanisms of surgical control of type 2 diabetes: GLP-1 is key factor *Surg Obes Relat Dis* 12(6):1236-42
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14. Guideline on Bioanalytical method validation, European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP), EMEA/CHMP/EWP/192217/2009, 21 July 2011

Further references can be found on our website: **www.mercodia.com**

Notes

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

Experiment: **Date:** **KIT LOT#:**

Summary of protocol sheet
Northern Lights Mercodia Total GLP-1 NL-ELISA

Add Calibrators, controls* and samples	25 µL
Add enzyme conjugate 1X solution	50 µL
Incubate	2 hours at 18-25°C on a plate shaker 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add substrate working solution	100 µL
Incubate	15 minutes in the dark at 18-25°C
Read chemiluminescence	1 s integration time (glow)

*not included

For full details see page 7

For technical support please contact: support@mercodia.com

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