Glucagon-Like Peptide-1 (Active)

96-Well Plate

Cat. # EGLP-35K

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Glucagon-Like Peptide-1 (Active) ELISA Kit
96-Well Plate (Cat. # EGLP-35K)

I. INTENDED USE

This kit is for non-radioactive quantification of biologically active forms of Glucagon-Like Peptide-1 [i.e. GLP-1 (7-36 amide) and GLP-1 (7-37)] in plasma and other biological media. It is highly specific for the immunologic measurement of active GLP-1 and will not detect other forms of GLP-1 (e.g., 1-36 amide, 1-37, 9-36 amide, or 9-37). The GLP-1 sequence is highly conserved between the species, with no sequence variation occurring at all in mammals. One kit is sufficient to measure 39 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF PROCEDURE

This assay is based, sequentially, on: 1) capture of active GLP-1 from sample by a monoclonal antibody, immobilized in the wells of a microwell plate, that binds specifically to the N-terminal region of active GLP-1 molecule, 2) washing to remove unbound materials, 3) binding of an anti GLP-1-alkaline phosphatase detection conjugate to the immobilized GLP-1, 4) washing off unbound conjugate, and 5) quantification of bound detection conjugate by adding MUP (methyl umbelliferyl phosphate) which in the presence of alkaline phosphatase forms the fluorescent product umbelliferone. Since the amount of fluorescence generated is directly proportional to the concentration of active GLP-1 in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of active GLP-1.

III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well microtiter plate and contains the following reagents:

A. GLP-1 (Active) ELISA Plate
Coated with anti-GLP-1 Monoclonal Antibody
Quantity: 1 plate
Preparation: Ready to use

B. Adhesive Plate Sealer
Quantity: 1 Sheet
Preparation: Ready to use

C. 10X Wash Buffer Concentrate
10X concentrate of 10 mM PBS Buffer containing Tween 20 and Sodium Azide.
Quantity: 50 mL
Preparation: Dilute 1:10 with deionized water

III. REAGENTS SUPPLIED (continued)

D. GLP-1 (7-36) amide ELISA Standards
GLP-1 (7-36 amide) in Assay Buffer: 2, 5, 10, 20, 50 and 100 pM
Quantity: 1 mL/vial
Preparation: Ready to use

E. ELISA GLP-1 (Active) Quality Controls 1 and 2
Various peptides including GLP-1 (7-36 amide) in QC Buffer.
Quantity: 1 mL/vial
Preparation: Ready to use

F. GLP-1 (Active) Assay Buffer
0.05M PBS, pH 6.8, containing proprietary protease inhibitors, with Tween 20, 0.08% Sodium Azide and 1% BSA.
Quantity: 25 mL
Preparation: Ready to use

G. GLP-1 (Active) Detection Conjugate
Anti GLP-1-Alkaline Phosphate Conjugate.
Quantity: 21 mL
Preparation: Ready to use

H. Substrate (Light sensitive, avoid unnecessary exposure to light)
MUP
Quantity: 10 mg
Preparation**:** Hydrate in 1 mL deionized water just before use. Use at 1:200 dilution in substrate diluent (e.g. 100 µL hydrated substrate in 20 mL substrate diluent). Dilute fresh each time just before use. Undiluted substrate may be used within one week after hydration if stored at ≤ - 20ºC. Do not reuse diluted substrate.

I. Substrate Diluent (Light sensitive, avoid unnecessary exposure to light)
Quantity: 21 mL
Preparation: Ready to use

J. Stop Solution
Quantity: 6 mL
Preparation: Bring to room temperature before use. Mix thoroughly to ensure no precipitate remains.

IV. STORAGE AND STABILITY

All components of the kit should be stored at ≤ -20°C. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

V. REAGENT PRECAUTIONS

A. Diethanolamine
Substrate diluent contains diethanolamine. This compound can be harmful through ingestion, inhalation, and skin contact. May be irritating to eyes and skin. If skin/eye contact occurs flush thoroughly with water.

B. Sodium Azide
Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and l ocal regulations.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipet with Tips, 10µL-200µL
2. Multi-channel Pipette, 50µL-300µL
3. Reagent Reservoirs
4. Vortex mixer
5. Refrigerator
7. Deionized Water
8. Fluorescence Plate Reader
9. DPP-IV Inhibitor (Millipore Cat# DPP4 recommended)

VII. SAMPLE COLLECTION AND STORAGE

1. For plasma collection, collect blood in ice-cooled Vacutainer® EDTA-plasma tubes. **Immediately (< 30 seconds)** after collection, add appropriate amount of DPP-IV inhibitor according to manufacturer's directions. Invert tube to mix and store tubes in ice bath. (If using Millipore Cat # DPP4, add 10 µl DPP-IV inhibitor per milliliter of blood.) Centrifuge immediately at 1000 xg for 10 minutes in refrigerated centrifuge or place tubes on ice and centrifuge within one hour.

2. Specimens can be stored at 4°C if they will be tested within 3 hours of collection. For longer storage, specimens should be stored at -70°C. Avoid multiple (>3) freeze/thaw cycles. Aliquot samples before freezing if necessary.

3. Avoid using samples with gross hemolysis or lipemia.

VIII. ASSAY PROCEDURE

The assay should be run in duplicate in a 200µl total volume.

First Day

1. Add 300µL diluted Wash Buffer (for preparation refer to Section III C) in each well. Incubate at room temperature for 5 minutes. Decant and tap out excess buffer on absorbent towels.
2. Add 200µL Assay Buffer to NSB (non-specific binding) wells A1, A2. Refer to Section IX for suggested microtiter plate arrangement.
3. Add 100µL Assay Buffer to the remaining wells.
4. Add 100µL standards in ascending order to wells A3, A4, etc.
5. In the next set of wells, add 100µL QC 1 (wells B3 and B4) and QC 2 (wells B5 and B6).
6. Add 100µL samples in the remaining wells. Shake plate gently for proper mixing.
7. Cover the plate with plate sealer. Incubate overnight (20 to 24 hours) at 4°C.

Second Day

8. Decant liquid from plate and tap out excess fluid on absorbent towels.
9. Wash the plate 5 times with 300µL Wash Buffer per well with 5-minute incubation at room temperature in Wash Buffer with the fourth wash. Tap out excess buffer on absorbent towels after the fifth wash.
10. Immediately add 200µL Detection Conjugate in each well. Incubate 2 hours at room temperature. Decant.
11. Wash 3 times with 300µL diluted Wash Buffer. Tap out excess buffer on absorbent towels.
12. Add 200µL diluted Substrate (for preparation, refer to Section III H) in each well. Incubate at least 20 minutes at room temperature in the dark. Monitor to see if there is significant signal-to-noise ratio with the lowest point on standard curve (i.e. 2 pM), and the highest standard point (i.e. 100 pM) within the maximum relative fluorescence unit (RFU) read-out of plate reader. Incubate longer if necessary.
13. If sufficient fluorochrome has been generated, add 50µL Stop Solution to each well in the same order as the Substrate was added. Incubate 5 minutes at room temperature in the dark to arrest phosphatase activity.
14. Read plate on a fluorescence plate reader with an excitation/emission wavelength of 355 nm/460 nm.

Assay Procedure for Glucagon-Like Peptide-1 (Active) ELISA Kit (Cat. # EGLP-35K)

	Step 1	Step 2-3	Step 4-6	Step 7-9	Step 10	Step 10-11	Step 12	Step 12	Step 13	Step 14
Well #	Add 300 µLWash Buffer to plate and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels.	Assay Buffer	Standards/ Controls/ Samples	Seal and Incubate overnight hour at 4°C. Wash 5X with 300 µL Wash Buffer with a 5 minute incubation at room temperature in Wash Buffer with the fourth wash. Remove residual buffer by tapping smartly on absorbent towels.	Detection Conjuga	Seal, Incubate 2 hours at Room Temperature . Wash 3X with 300 µL Wash Buffer	Substrate	Seal, Incubate at least 20 minutes at Room Temperature in the dark.	Stop Solution	Read fluorescence at 355 nm/460 nm.
A1, A2		200 µL	----------		200 µL		200 µL		50 µL	
A3, A4		100 µL	100 µL of 2 pM Standard		200 µL		200 µL		50 µL	
A5, A6		100 µL	100 µL of 5 pM Standard		200 µL		200 µL		50 µL	
A7, A8		100 µL	100 µL of 10 pM Standard		200 µL		200 µL		50 µL	
A9, A10		100 µL	100 µL of 20 pM Standard		200 µL		200 µL		50 µL	
A11, A12		100 µL	100 µL of 50 pM Standard		200 µL		200 µL		50 µL	
B1, B2		100 µL	100 µL of 100 pM Standard		200 µL		200 µL		50 µL	
B3, B4		100 µL	100 µL of QC 1		200 µL		200 µL		50 µL	
B5, B6		100 µL	100 µL of QC 2		200 µL		200 µL		50 µL	
B7, B8 ↓		100 µL	100 µL of Sample		200 µL		200 µL		50 µL	

IX. MICROTITER PLATE ARRANGEMENT

Standard Glucagon-Like Peptide-1 (Active) ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	2 pM	2 pM	5 pM	5 pM	10 pM	10 pM	20 pM	20 pM	50 pM	50 pM
B	100 pM	100 pM	QC 1	QC 1	QC 2	QC 2	Sample 1	Sample 1	Sample 2	Sample 2	etc….	
C												
D												
E												
F												
G												
H												

X. CALCULATIONS

The RFU can be fitted directly to the concentration. If curve fitting software is available, the best fit can be obtained with a linear-linear spline fit.

Since this assay is a direct ELISA, the RFU is directly proportional to the concentration of GLP-1 in the sample.

XI. ASSAY CHARACTERISTICS

Sensitivity

The lowest level of GLP-1 that can be detected by this assay is 2 pM (100 µl plasma sample size).

Performance

ED_{80} = 81 ± 4 pM
ED_{50} = 55 ± 7 pM
ED_{20} = 28 ± 1 pM

Crossreactivity

GLP-1 (7-36 amide)	100%
GLP-1 (7-37)	100%
GLP-1 (9-36 amide)	ND
GLP-2	ND
Glucagon	ND

ND- Not Detectable

XI. ASSAY CHARACTERISTICS (continued)

Precision

Within and Between Assay Variation

Sample No.	Mean pM	Within % CV	Between % CV
1	4	8	13
2	8	7	12
3	12	6	7
4	28	7	7
5	76	9	<1

Within and between assay variation was performed on five human plasma samples containing varying concentrations of GLP-1. Data shown are from four duplicate determinations for within and four duplicate determinations for between.

Recovery

Spike & Recovery of GLP-1 in Human Plasma

Sample #	GLP-1 added pM	Expected pM	Observed pM	% of Recovery
1	0	5	5	100
	10	15	13	87
	20	25	21	84
	50	55	43	78
2	0	13	13	100
	10	23	20	87
	20	33	29	88
	50	63	50	80
3	0	12	12	100
	10	22	20	91
	20	32	28	88
	50	62	53	85
4	0	37	37	100
	10	47	44	94
	20	57	55	96
	50	87	74	85

Varying concentrations of GLP-1 were added to four human plasma samples and the GLP-1 content was determined by ELISA. Mean of the observed levels from four duplicate determinations are shown.

Percent recovery = observed ÷ expected x 100%.

XI. ASSAY CHARACTERISTICS (continued)

Linearity

Effect of Plasma Dilution

Sample No.	Volume Sampled	Expected pM	Observed pM	% Of Expected
1	100 µL	5	5	100
	50 µL	3	3	100
	25 µL	< 2	< 2	---
2	100 µL	13	13	100
	50 µL	7	7	100
	25 µL	3	4	133
3	100 µL	11	11	100
	50 µL	6	7	116
	25 µL	3	4	133

Aliquots of pooled human plasma containing varying concentrations of GLP-1 were analyzed in the volumes indicated. The mean GLP-1 levels and percent of expected from four duplicate determinations are shown.

XII. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com/techlibrary/index.do

XIII. TROUBLESHOOTING GUIDE

Low or No Signal with Standards

* Standards were left at room temperature. Standards should be stored at ≤ -20ºC.
* Insufficient time for reaction with substrate. Allow substrate to react longer.
* Kit reagents have expired.
* Inadequate plate washing after sample incubation.
* Too much washing after conjugate incubation can however reduce signal.

High Background

* Inadequate plate washing. After conjugate incubation, tap out plate on absorbent towels after decanting.
* Plate was not kept in dark after substrate addition.
* Cross contamination between neighboring wells.
* Substrate has been diluted too long or exposed to light before use, or diluent has been contaminated with old substrate. Check only substrate in a well.

Samples too High

* Dilute sample 1:10 with assay buffer to bring GLP-1 concentration within standard range.

Signal too High on Highest Standard

* Plate incubated too long with substrate. Discard substrate, wash plate once and add freshly prepared substrate. Check RFU in less time.

High Variance in RFU of Duplicates

* Cross contamination in wells
* Bubbles in substrate at time of reading
* Loss of reagent or faulty pipetting in duplicates

XIV. REPLACEMENT REAGENTS

Reagents	**Cat. #**
GLP-1 (Active) ELISA Plate	EP35
10X Wash Buffer Concentrate	EWB
GLP-1 (7-36) amide ELISA Standards	E8035-K
ELISA GLP-1 (Active) Quality Controls 1 & 2	E6016-K
GLP-1 (Active) Assay Buffer	AB-GLPHK
GLP-1 (Active) Detection Conjugate	E1035
Substrate	ESS-MUP
Substrate Diluent	EDD-MUP
Stop Solution	ET-AP
10-pack of Glucagon-Like Peptide-1 (Active) ELISA kits	EGLP-35BK

XV. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, email, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

TELEPHONE ORDERS:
Toll Free US (800) MILLIPORE

FAX ORDERS: (636) 441-8050
MAIL ORDERS: EMD Millipore Corporation
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers, it is Millipore's policy to sell our products only through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

B. Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.

XVI. REFERENCES

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