DRG GLP-1 ELISA (Glucagon-Like Peptide-1) (EIA-3056)

Revised 30 Aug. 2010 rm (Vers. 3.1) **RUO**

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

This kit is for determination 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 purposes only.

2 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.

3 REAGENTS SUPPLIED

Each kit is sufficient to run one 96 well microtiter plates 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

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.

4 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.

5 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 has been added to reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

6 MATERIALS REQUIRED BUT NOT PROVIDED

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

7 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 Cat # DPP4, add 10 µl DPP-IV inhibitor per milliliter of blood.)
   Centrifuge immediately at 1000 x g 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.

8 ASSAY PROCEDURE

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

First Day

1. Add 300 µl diluted Wash Buffer (for preparation refer to Section 3.C) in each well.
   Incubate at room temperature for 5 minutes. Decant and tap out excess buffer on absorbent towels.
2. Add 200 µl Assay Buffer to NSB (non-specific binding) wells A1, A2. Refer to Section 9 for suggested microtiter plate arrangement.
3. Add 100 µl Assay Buffer to the remaining wells.
4. Add 100 µl Standards in ascending order to wells A3, A4, etc.
5. In the next set of wells, add 100 µl QC 1 (wells B3 and B4) and QC 2 (wells B5 and B6).
6. Add 100 µl 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 300 µl 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 200 µl Detection Conjugate in each well.
    Incubate 2 hours at room temperature. Decant.
11. Wash 3 times with 300 µl Wash Buffer. Tap out excess buffer on absorbent towels.
12. Add 200 µl diluted Substrate (for preparation, refer to Section 3.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 50 µl 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

	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 µL Wash 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 Conjugate	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	

9 MICROTITER PLATE ARRANGEMENT

	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												

10 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.

11 QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert.

12 TROUBLESHOOTING GUIDE

Low or No Signal with Standards

- Standards were left at room temperature. Standards should be stored at ≤-20°C.
- 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

13 ORDERING INFORMATION

Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for in vitro use only.

Material Safety Data Sheets (MSDS)

Material safety data sheets may be ordered by fax or phone.

14 REFERENCES / LITERATURE

1. Nathan DM, Schreiber E, Fogel H, Mojsov S, Hebener JF. Insulinotropic Action of Glucagon-like peptide-1 (7-37) in Diabetic and Nondiabetic Subjects. Diabetes Care 15: 270-276, 1992
2. Kieffer TJ, McIntosh CHS, Pederson RA. Degradation of Glucose-Dependent Insulinotropic Polypeptide (GIP) and Truncated Glucagon-Like Peptide (GLP-1) in vitro and in vivo by Dipeptidyl Peptidase IV. Endocrinology 136: 3585-3596, 1995
3. Tijsen P. "Practice and Theory of Enzyme Immunoassays" in Burdon RH and Knippenberg PH (Ed.), Laboratory Techniques in Biochemisrty and Molecular Biology. Amsterdam/NY: Elsevier, 1985
4. Christopoulos TK and Diamandis EP. "Fluorescence Immunoassays" in Diamandis EP and Christopoulos TK (Ed.), Immunoassay. Academic Press, 1996
5. Holst JJ, Cathrin Orskov, Bolette Hartmann, Carolyn F. Deacon : Postranslational processing of proglucagon and postsecretory fate of proglucagon products; in Fehmann HC, Goke B (eds) : The Insulinotropic Gut Hormone Glucagon-Like-Peptide-1. Front Diabetes. Basel, Karger, 1997, vol 13, pp 24-48