Lixisenatide ELISA

For the quantitative determination of Lixisenatide in human serum, plasma, and cell culture supernatant.

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

Store Human GLP-1 Antibody HRP Conjugate Concentrate at -20°C.

Catalog Number: 07-LIXHU-E01
Size: 96 determinations
Version: 2.1 **ALPCO**: 1.0

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INTENDED USE

The Lixisenatide ELISA is used as for the quantitative determination of Lixisenatide in human serum, plasma, and cell culture supernatant. For Research Use Only. Not for use in diagnostic procedures.

INTRODUCTION

Lixisenatide is a glucagon-like peptide-1 (GLP-1) receptor agonist used in the treatment of type 2 diabetes mellitus (T2DM). It is sold under the brand name Adlyxin by Sanofi-Aventis.

PRINCIPLE OF THE ASSAY

The method employs the quantitative sandwich enzyme immunoassay technique. Human GLP-1 antibody is pre-coated onto microwells. Samples and standards are pipetted into microwells and Lixisenatide present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Human GLP-1 antibody is pipetted and incubated. After washing microwells to remove any non-specific binding, the ready-to-use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Lixisenatide in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

MATERIALS SUPPLIED

Part	Description	Qty	Preparation
Human GLP-1 Antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human GLP-1 antibody	1 x 96 wells	Ready-to-Use
Lixisenatide Standard	Lixisenatide in a Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane – lyophilized (20,000 ng/ml)	2 vials	Lyophilized, see Reagent Preparation
Human GLP-1 Antibody HRP Conjugate Concentrate	Human GLP-1 antibody conjugated to Horseradish Peroxidase concentrated (1 mg/ml). *Store at -20°C upon receipt.*	1 vial	See Reagent Preparation
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	12 ml	Ready-to-Use
Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	2 x 50 ml	Ready-to-Use
Standard Diluent	Buffered protein base with 1:1000 dilution human serum and protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	10 ml	Ready-to-Use
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thimerosal <0.01%. May turn yellow over time.	25 ml	See Reagent Preparation
TMB Substrate	Stabilized Chromogen. Light-sensitive.	12 ml	Ready-to-Use
Stop Solution	0.73M Phosphoric Acid	12 ml	Ready-to-Use
Instruction Manual		1 no	

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ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter Plate Reader able to measure absorbance at 450 nm.
- Adjustable pipettes to measure volumes ranging from 25 ul to 1000 ul
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Graph paper or software for data analysis
- Tubes for standard/sample dilutions
- Absorbent paper
- 37°C incubator
- Centrifuge
- Timer

PRECAUTIONS

- This kit is for Research Use Only. Follow the work instructions carefully.
- The expiration dates stated on the kit must be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shifts during pipetting of reagents.
- All reagents should be kept in the original shipping containers.
- Some of the reagents contain a small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to touch skin or mucosa.

- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all samples as if potentially hazardous.

- Since the kit contains potentially hazardous materials, the following precautions should be observed
    - Do not smoke, eat, or drink while handling kit material
    - Always use protective gloves
    - Never pipette material by mouth
    - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case Good Laboratory Practice should be applied with all general and individual regulations to the use of this kit.
- Reagents that contain preservatives may be harmful if ingested, inhaled, or absorbed through the skin. Refer to SDS for more details.

REAGENT HANDLING and STORAGE CONDITIONS

1. Aliquot and store the Anti-GLP-1 HRP Conjugate concentrate at **-20°C** upon receipt. Immediately discard any excess 1x Working Anti-GLP-1 HRP Conjugate after running the assay.
2. Store the rest of the kit components at 2-8°C.
3. Use all reagents and wash solutions within 12 months of manufacturing date.
4. Before use, bring all components to room temperature (18-25ºC).
5. Upon assay completion, ensure all kit components are returned to appropriate storage conditions.
6. The substrate is light-sensitive and should be protected from direct sunlight or UV sources.

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SAMPLE COLLECTION, PREPARATION, AND STORAGE

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma may also be used. Avoid lipemic, hemolytic, or contaminated samples. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, aliquot samples and keep at -20°C.

Serum

Samples must be diluted 1:1000 (v/v), e.g. for 1:1000 (1 ul sample + 999 ul sample diluent) prior to assay. The samples may be kept at 2-8°C for up to three days. Long-term storage requires -20°C.

Cell Culture Supernatant

If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80º C. Avoid repeated freeze-thaw cycles.

Allow samples to reach room temperature prior to assay. Gently agitate samples to ensure homogeneity.

REAGENT PREPARATION

All reagents should be diluted immediately prior to use.

1. Label any aliquots made with the kit Lot Number and Expiration Date and store as directed.
2. Bring all reagents to room temperature before use.
3. To prepare **1x working Wash Buffer**; dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.
4. **Standards Preparation**
   Reconstitute the concentrated Standard lyophilized vial with 250 ul of Standard Diluent to obtain a concentration of 20,000 ng/ml. Agitate the vial gently for 15 mins before making further dilutions. Dilute 240 ul of Reconstituted Standard **(20,000 ng/ml)** with 160 ul of Standard Diluent to generate a **12000 ng/ml Standard Solution**. Prepare more **Standards** by serially diluting the Standard Solution as per the table below. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Preparation Instructions
20,000 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 250 ul Standard Diluent (1X)
12000 ng/ml	Standard No.7	240 ul Reconstituted Standard + 160 ul Standard Diluent (1X)
6000 ng/ml	Standard No.6	200 ul Standard No.7 + 200 ul Standard Diluent (1X)
3000 ng/ml	Standard No.5	200 ul Standard No.6 + 200 ul Standard Diluent (1X)
1500 ng/ml	Standard No.4	200 ul Standard No.5 + 200 ul Standard Diluent (1X)
750 ng/ml	Standard No.3	200 ul Standard No.4 + 200 ul Standard Diluent (1X)
375 ng/ml	Standard No.2	200 ul Standard No.3 + 200 ul Standard Diluent (1X)
187.5 ng/ml	Standard No.1	200 ul Standard No.2 + 200 ul Standard Diluent (1X)
ng/ml	Standard No. 0	Only Standard Diluent (1X)

Note: Use standards immediately upon reconstitution. Discard leftovers.

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5. Human GLP-1 Antibody HRP Conjugate Working Solution Preparation:
See **Reagent Preparation sheet attached to Certificate of Analysis (CoA**) for lot-specific instructions (enclosed in the kit).

QUALITY CONTROL

It is recommended that each laboratory test appropriate quality control samples with each run to ensure that all reagents and procedures are correct.

PROCEDURAL NOTES

1. To achieve good assay reproducibility and sensitivity, proper washing to remove excess reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Lixisenatide. High Dose Hook Effect is due to excess of antibody for very high concentrations of Lixisenatide present in the sample. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. If the Lixisenatide concentration of the undiluted sample is less than the diluted sample, this may be indicative of Hook Effect.
3. Avoid using samples containing sodium azide (NaN3), as it could destroy the HRP activity resulting in underestimation of the amount of Lixisenatide.
4. It is recommended that all standards and samples be run in duplicate.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are the same for each well.
6. If the Substrate has a distinct blue color prior to use, it may be contaminated, and use of such substrate can lead to inaccurate results.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a plate map to identify the location of standards and samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be run in duplicate. A standard curve is required for each assay. All incubation steps are at 37°C.

1. Add **100 ul** of prepared **Standards** or diluted **Samples** into the respective wells.
2. Cover the plate and incubate for 120 minutes at 37°C.
3. Aspirate and wash plate 4 times with 300 – 350 µL **1x working Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
4. Pipette **100 ul** of **1x Working Human GLP-1 antibody HRP Conjugate** into each well.
5. Cover the plate and incubate for 120 minutes at 37°C.
6. Aspirate and wash plate 4 times with 300 – 350 µL **1x working Wash Buffer** as mentioned in **Step 4.**
7. Add **100 ul** of **TMB Substrate** in each well.
8. Incubate the plate at **37°C** for 30 minutes in dark. **DO NOT SHAKE**. Shaking may result in higher backgrounds and poor precision. Positive wells should turn bluish in color.
9. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
10. Read the absorbance at 450 nm with a microplate reader.

CALCULATION OF RESULTS

Determine the Mean Absorbance for each set of duplicate standards and samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the

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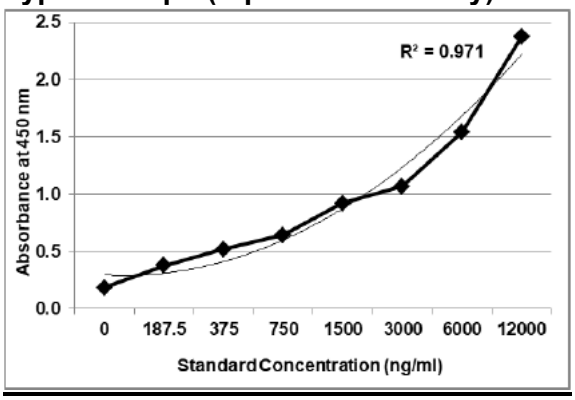
standard points. To determine the unknown Lixisenatide concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Lixisenatide Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software capable of generating a cubic spline curve-fit or a 4PL polynomial curve (2nd order) is recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard the assay should be repeated with a lower dilution factor.
- If the absorbance value is equivalent or higher than the 12,000 ng/ml standard, the assay should be repeated with a higher dilution factor.

Typical Data (representative only)

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.186	--	--
187.5	0.378	158.3	84.4
375	0.518	448.4	119.6
750	0.643	820.8	109.4
1500	0.920	1795.2	119.7
3000	1.068	2617.2	87.2
6000	1.542	5497.0	91.6
12000	2.376	12426.8	103.6

Typical Graph (representative only)



PERFORMANCE CHARACTERISTICS

This kit was validated per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:

Limit of Detection:
It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD. 10 replicates of '0' standards were evaluated and the LOD was ~180 ng/ml.

Specificity:

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The antibodies used in the kit are monoclonal antibodies, anti-idiotypic, and specific for Lixisenatide peptide. There is cross-reactivity observed between GLP-1 analogs like Exenatide, Semaglutide, and Liraglutide which has not been validated with this assay.

Standards provided in the kit will be used for measuring the linearity range of Lixisenatide present in the matrix.

Precision:

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (187.5 ng/ml), medium (3000 ng/ml) and high (12000 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	**Intra Assay %CV**	**Inter Assay %CV**
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

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