Exenatide ELISA

For the quantitative determination of Exenatide in human serum and plasma.

Store standards frozen at -20° C.

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

Catalog Number: 07-EXEHU-E01
Size: 96 determinations
Version: 2.0 **ALPCO**: 1.0

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INTENDED USE

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INTRODUCTION

Exenatide or Extendin-4 is a potent GLP-1 receptor agonist. Exenatide (marketed as Byetta) is one of a new class of medications (incretin mimetics) approved (in April 2005) for the treatment of diabetes mellitus type 2.

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 exenatide present in the sample is bound by the capture antibody. Then, an 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 Exenatide in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

MATERIALS SUPPLIED

07-EXEHU-E01			
Component	**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
Exenatide Standard	Exenatide in a buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane – lyophilized (10 ug/ml)	2 vials	See Reagent Preparation
Human GLP-1 antibody HRP Conjugate	Human GLP-1 antibody conjugated to Horseradish Peroxidase with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	1x 12 ml	Ready-to-Use
(1X) Standard Diluent	Buffered protein base with 1:1000 dilution human serum and protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	1 x 10 ml	Ready-to-Use
(1X) Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	2 x 50 ml	Ready-to-Use
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	1 x 25 ml	See Reagent Preparation
TMB Substrate	Stabilized Chromogen	1 x 12 ml	
Stop Solution	0.73M Phosphoric Acid	1 x 12 ml	
Plate Sealers		2	
Instruction Manual		1	

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter Plate Reader able to measure absorbance at 450 nm.
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
- Deionized (DI) water

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- Wash bottle or automated microplate washer
- Polypropylene vials to aliquot and store standards if necessary
- Graph paper or software for data analysis
- Absorbent paper
- Timer
- 37°C incubator

PRECAUTIONS

- This kit is for Research Use Only. Follow the work instructions carefully.
- Reagents that contain preservatives may be harmful if ingested, inhaled, or absorbed through the skin.
- 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 come into contact with 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. Store all kit components at 2-8°C.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before use, bring all components to room temperature (18-25ºC).
4. Upon assay completion, ensure all kit components are returned to appropriate storage conditions.
5. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

SAMPLE COLLECTION, PREPARATION, AND STORAGE

Allow samples to reach room temperature prior to assay. Agitate samples gently to ensure homogeneity.

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Serum and Plasma
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 Sample Preparation:
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 -80ºC. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

All reagents should be diluted immediately prior to use.

1. Label any aliquots made with the kit Lot No. and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to room temperature before use.
3. To make **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 500 ul of Standard Diluent to obtain a concentration of 10,000 ng/ml. Let sit for 15 mins with gentle agitation before making further dilutions. Dilute 800 ul of original **Standard (10,000 ng/ml)** with 200 ul of Standard Diluent to generate an **8000 ng/ml Standard Solution**. Prepare further **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	Dilution Particulars
10,000 ng/ml	Original Standard	Original Standard provided in the Kit +500 ul Standard Diluent (1X)
8000 ng/ml	Standard No.7	800 ul Original Standard (10,000 ng/ml) + 200 ul Standard Diluent (1X)
4000 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
2000 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
1000 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
500 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
250 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
125 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No. 0	Only Standard Diluent

Note: Use the Standards as soon as possible upon reconstitution. Discard any leftovers.

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

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PROCEDURAL NOTES

1. To achieve good assay reproducibility and sensitivity, proper plate washing is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Exenatide. High Dose Hook Effect is due to excess of antibody for very high concentrations of Exenatide present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is suspected, the samples to be assayed should be diluted with a compatible diluent. Thus, if the Exenatide concentration of the undiluted sample is less than the diluted sample, this may be indicative of Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Exenatide.
4. It is recommended that all Standards and Samples be assayed in duplicate.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation time is the same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can compromise the sensitivity of the assay.
7. The plates should be read within 30 minutes of adding the Stop Solution.
8. Make a work list to identify the location of Standards and Samples.

ASSAY PROCEDURE

Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicate or triplicate. A standard curve is required for each assay. All incubation steps must be performed 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 the plate 4 times with 300 µL / well of **1x working Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as residue can interfere in the reading step.

4. Pipette **100 ul** of **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 the plate 4 times with 300 µL / well of **1x working Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as residue can interfere in the reading step.

7. Add **100 ul** of **TMB Substrate** in each well.

8. Incubate the plate at **37°C** for 30 minutes in the dark. **DO NOT SHAKE** or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

9. Add **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

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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 curve fit through the standard points. To determine the unknown Exenatide 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 Exenatide Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which can generate a cubic spline curve-fit or a 4PL polynomial curve (2nd order) is recommended for automated results.

Note:

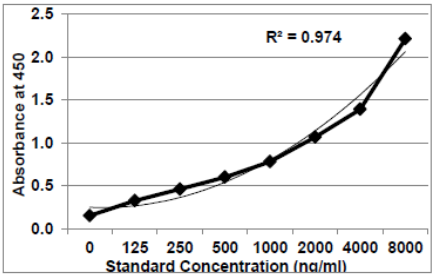
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 8000 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Means Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.155	--	--
125	0.329	100.8	80.6
250	0.463	297.5	119.0
500	0.602	584.1	116.8
1000	0.783	1067.0	106.7
2000	1.070	2059.1	103.0
4000	1.394	3478.3	87.0
8000	2.215	8322.9	104.0

Typical Standard Curve

Example standard curve.



PERFORMANCE CHARACTERISTICS

This kit has been validated as 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 found to be ~220 ng/ml.

Specificity:

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

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Exenatide present in 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 (250 ng/ml), medium (2000 ng/ml) and high (8000 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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