Version 23-05/24

User's Manual

Liraglutide High Sensitivity ELISA Kit

REF **DEIA-XYZ95**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of liraglutide in serum, plasma.

General Description

Liraglutide is an acylated glucagon-like peptide-1 (GLP-1) agonist, derived from human GLP-1-(7-37), a less common form of endogenous GLP-1. It reduces meal related hyperglycemia, delaying gastric emptying, and suppressing prandial glucagon secretion. Liraglutide is sold as a medication used to treat diabetes mellitus type 2 and obesity.

The prolonged action of liraglutide (as compared to endogenous GLP-1) is achieved by attaching a fatty acid molecule at one position of the GLP-1-(7-37) molecule, enabling it to both self-associate and bind to albumin within the subcutaneous tissue and bloodstream. The active GLP-1 is then released from albumin at a slow, consistent rate.

This ELISA was developed with serum from rabbits immunized with Liraglutide coupled to a carrier protein.

Immunogen: Synthetic peptide H-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-(γ-Glu-palmitoyl)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH coupled to carrier protein.

Principles of Testing

This ELISA kit is a competitive immunoassay. The anti-Liraglutide IgG antibodies in the antiserum are captured by anti rabbit IgG antibodies coated on a 96-well plate. A constant concentration of biotinylated tracer (Bt-tracer) and varying concentrations of unlabeled standard or sample peptide compete for binding specifically to the anti-Liraglutide. Captured Bt-tracer is subsequently bound by streptavidin-conjugated horseradish peroxidase (SA-HRP), which produces a soluble colored product after a substrate is added.

The sequence of the antigen is shown on the datasheet.

Make a standard curve in the range specified in the kit's datasheet. Standard curves are S-shaped (on a semi-log plot) or almost linear over the kit's range. The measuring range is the range of standard concentrations near the middle or near the IC50 (15 ng/ml) of the standard curve. Unknown sample concentrations are measured by comparing their absorbance with the standard curve.

We include sufficient reagents for 96 determinations.

Reagents And Materials Provided

1. Wash Buffer (50 ml, 20 × concentrate)
2. 96-well immunoplate with plate sealer
3. Antiserum (lyophilized powder)
4. Biotinylated tracer (lyophilized powder)
5. Streptavidin-HRP (12 ml, ready to use)

6. Sample Diluent (50 ml, ready to use)
7. TMB substrate (2 × 6 ml, ready to use)
8. Stop solution (7 ml, ready to use)
9. Kit Datasheet/Protocol Insert

Materials Required But Not Supplied

1. Standard Peptide. Purchase synthetic peptide with sequence identical to the antigen sequence listed on the datasheet
2. 96-well microtiter plate reader set up to measure 450nm and 620nm
3. 96-well plate washer and shaker (optional)
4. Distilled, deionized or USP water
5. Curve fitting software (optional)
6. Test tubes, pipettes and various other standard laboratory items

Storage

After you receive the kit, all the components should be stored in the refrigerator (4-8°C) also up to 1 year. Long term storage, improper storage conditions and large temperature fluctuation cycles may cause precipitates in the TMB solution. These precipitates should not affect the assay noticeably. Nevertheless, if you observe such precipitates, we recommend to avoid them by allowing them to sink to the bottom.

Specimen Collection And Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

Serum: Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.

Notes:

The concentration of the target molecule must be within the measuring range of the kit. If the concentration range of your sample is difficult to estimate, prepare it at different concentrations such that one of the samples should lie within the measuring range.

Plate Preparation

Seven-Point Standard Curve Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk		U1		U9		U17		U25		U33	
B	S1		U2		U10		U18		U26		U34	
C	S2		U3		U11		U19		U27		U35	
D	S3		U4		U12		U20		U28		U36	
E	S4		U5		U13		U21		U29		U37	
F	S5		U6		U14		U22		U30		U38	
G	S6		U7		U15		U23		U31		U39	
H	S0		U8		U16		U24		U32		U40	

Blk = blank S = standards U = unknown samples

* All standards and samples are run in duplicates

Reagent Preparation

Lyophilized kit components should not be re-hydrated until they are needed. Please read the complete protocol before proceeding.

1. Equilibrate unopened kit components to room temperature. Avoid accumulation of moisture, do not open reagents and immunoplate while they are cold.
2. Wash buffer. Dilute the Wash buffer concentrate 1 in 20 with water and mix well.

   Example: mix the 50 ml contained in the kit with 950ml of water.
3. Antiserum. Add 3ml of Sample Diluent and vortex.
4. Biotinylated tracer. Add 5ml of Sample Diluent to the vial of lyophilized biotinylated peptide and vortex.
5. Standard:

Suggested Preparation of Standards		
	ng/ml	Range: 0.98 to 1000 ng/ml
Stock	1000	
S1	1000	Add 1000µl Stock + 0µl diluent
S2	250.00	Add 200µl S1 +600µl diluent
S3	62.50	Add 200µl S2 +600µl diluent
S4	15.63	Add 200µl S3 +600µl diluent
S5	3.91	Add 200µl S4 +600µl diluent
S6	0.98	Add 200µl S5 +600µl diluent
S0	0.00	500µl diluent

Assay Procedure

Procedure Note:

1. **Sample Diluent.** The antiserum and the Bt-tracer are reconstituted and used in Sample Diluent. The standard dilutions and samples are prepared in Sample Diluent.
2. **Room Temperature.** Reagents, samples, and the plate should be brought to room temperature before use.
3. **Shakers.** Shakers (optional) may help lower the experimental variation of duplicates (recommended at 60 rpm).
4. **Blank Wells.** Blanks will give you the background to be subtracted from all readings. These should not be

confused with the "S0 Standards" which contain no standard peptide and which will yield the highest readings. Blank readings will notinfluence concentration calculations - thus, they are optional.

Procedure

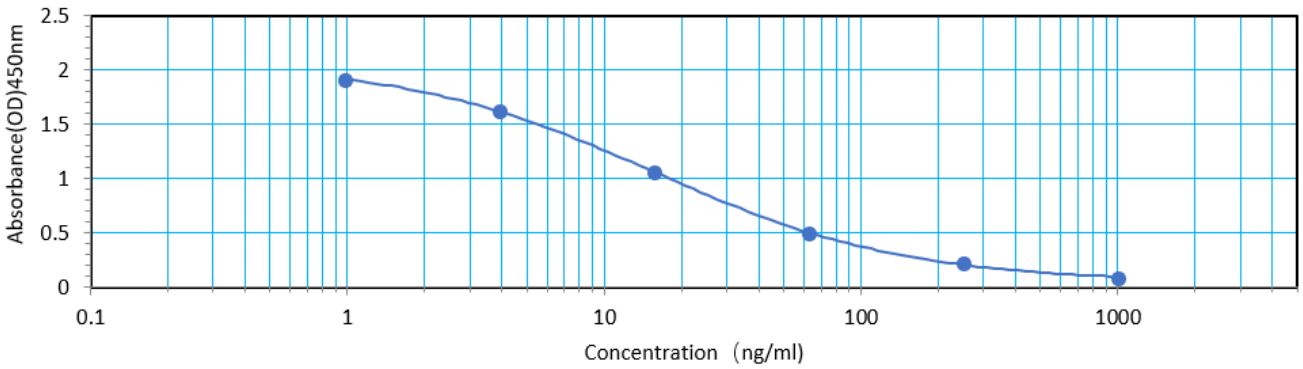
1. Into each well of the immunoplate, except blank wells, add 50ul standard or sample (in Sample Diluent); 25ul antiserum (in Sample Diluent); Add 75ul Sample Diluent to blank wells.
2. Incubate at room temperature for 1 hour. Shorter incubation may result in lower sensitivity.
3. Rehydrate the Bt-tracer (in Sample Diluent), add 25ul per well.
4. Incubate at room temperature for 2 hours.
5. Wash immunoplate 5 times with 300µl per well of Wash buffer. Be careful not to cross-contaminate between wells in the first wash/dispensing cycle. In each wash cycle empty plate contents with a rapid flicking motion of the wrist, then gently blot dry the top of plate on paper towels. Dispense 300 µl of Wash buffer into each well and gently shake for at least a few seconds. Thorough washing is essential.
6. Add 100 µl per well of streptavidin-HRP, including the blanks.
7. Incubate at room temperature for 1 hour.
8. Wash immunoplate 5 times (see step 5).
9. Add 100 µl per well of TMB solution, including the blanks.
10. Incubate at room temperature for 15 minutes. You may read the developing blue color at 620 nm and use the data for your calculations.
11. Terminate reactions by adding 50 µl Stop solution per well.
12. Read absorbance at 450 nm within ten minutes.

Calculation

Plot the standard curve on semi-log graph paper. Construct a standard curve by plotting the known concentrations of standard peptide on the log scale (X-axis), and its corresponding O.D. reading on the linear scale (Y-axis). It is strongly recommended to use curve-fitting software capable of 4 parameter logistics or log-logit to quantify the concentration of standard peptide. The standard curve shows an inverse relationship between peptide concentrations and the corresponding absorbance. As the standard concentration increases, the yellow color decreases, thereby reducing the O.D. absorbance.

The concentration of peptide in a sample is determined by locating the sample's O.D. on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line drawn from this point will intersect the X-axis at a coordinate corresponding to the peptide concentration in the sample. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors. The standard curve will be a reverse sigmoidal shape. Refer to QC Data Sheet for acceptable values of the positive controls.

Typical Standard Curve



	y=（A-D）/（1+（x/C＾B） + D：	A	B	C	D	R^2
●Std(Standards: Concentration vs MeanValue)		2.077	0.891	15.579	0.051	0.999

Detection Range

0-1000 ng/ml

Precautions

The kit's IC50, or the shape of the standard curve, may exhibit some variation but this will not affect the kit's accuracy in the measuring range. The kit accurately measures sample peptides if the following conditions are met.

1. Both samples and standards must be measured in the same diluent and under the same conditions (same microtiter plate).
2. The kit's antiserum must not cross-react appreciably with other factors present in the sample. Cross-reactivity tables are included with each kit. The user may wish to test the cross-reactivity with other peptides.
3. The sample peptides must be identical to the standard. Ideally, the synthetic standard mimics the natural peptide perfectly. Sometimes, however, natural peptides exist as families of species related by a common or similar sequence. In addition, natural peptides may be enzymatically or spontaneously modified, may exist in complexes, and may assume alternative structural forms. In these cases the kit might not measure the exact concentration of a particular natural peptide species, but it may still be used for relative average measurements.