Version 15-04/24

User's Manual

Human Exendin-4 ELISA Kit

REF **DEIABL227**

Σ **96T**

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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.

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PRODUCT INFORMATION

Intended Use

For the quantitative determination of Exendin-4 in human serum or plasma using competitive ELISA method.

General Description

Exendin-4 is a 39 amino acid peptide amide known to be a long-acting agonist of the glucagon-like peptide 1 receptor (GLP-1). It is involved in glucose-dependent enhancement of insulin secretion, reduction of hyperglycemia, and glucosedependent suppression of high glucagon secretion.

This ELISA was developed with serum from rabbits immunized with Exendin-4 coupled to a carrier protein.

Principles of Testing

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

Reagents And Materials Provided

1. ELISA buffer concentrate (50ml 20x concentrate)
2. 96-well immunoplate with plate sealer
3. Antiserum (lyophilized powder)
4. Standard (1ug lyophilized powder)
5. Biotinylated tracer (lyophilized powder)
6. Streptavidin-HRP (100µl 200x concentrate)
7. TMB substrate stock solution (1.5ml)
8. TMB substrate buffer (15ml citrate buffer)
9. Stop solution 2 N HCl (15 ml)
10. Standard diluent 8 ml (peptide-free human serum)
11. Datasheet
12. Protocols

Materials Required But Not Supplied

1. 96-well microtiter plate reader set up to measure 450nm and 650nm
2. 96-well plate washer and shaker (optional)
3. Distilled or deionized water, or comparable quality

4. Curve fitting software (optional)
5. Test tubes, pipettes and various other standard laboratory items

Storage

After you receive the kit, store the lyophilized components and standard diluent at -20°C for up to one year from the kit's assembly date. The remaining 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 and in the ELISA buffer concentrate. 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.

Plate Preparation

Seven-Point Standard Curve Layout

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

Blk = blank S = standards U = unknown samples

Reagent Preparation

Lyophilized kit components should not be re-hydrated until they are needed. Please check the included datasheet for the appropriate protocol.

1. Equilibrate unopened kit components to room temperature. Avoid accumulation of moisture, do not open reagents and immunoplate while they are cold.
2. ELISA buffer. Dilute the ELISA buffer concentrate 1 in 20 with water and mix well. Example: mix the 50ml contained in the kit with 950ml of water.
3. Standard. Add 1ml of standard diluent buffer [serum] to the vial of lyophilized standard peptide (1ug) and vortex gently. If samples are to be extracted and re-suspended in ELISA buffer as described below, use ELISA buffer as a diluent. Otherwise, we encourage customers to use their own diluent such that standards and samples will be treated equally. For extraction-free (EIAS) kits we provide peptide-free human serum as a diluent, but again, customers should use their own sample diluent provided it does not bind appreciably to the antiserum.
4. Standard curve. Make serial dilutions of the standard to cover the range of this kit.

Suggested Preparation of Standards		
	ng/ml	Range: 0.02 to 25ng/ml
Stock	1000	
S1	25.00	Add 25µl Stock + 975µl diluent
S2	6.25	Add 200µl S1 + 600µl diluent
S3	1.56	Add 200µl S2 + 600µl diluent
S4	0.39	Add 200µl S3 + 600µl diluent
S5	0.10	Add 200µl S4 + 600µl diluent
S6	0.02	Add 200µl S5 + 600µl diluent
S0	0.00	500µl diluent

5. Antiserum. Add 5ml of ELISA buffer and vortex.
6. Biotinylated tracer. Add 5ml of ELISA buffer to the vial of lyophilized biotinylated peptide and vortex. Please check the datasheet for exceptions.

Assay Procedure

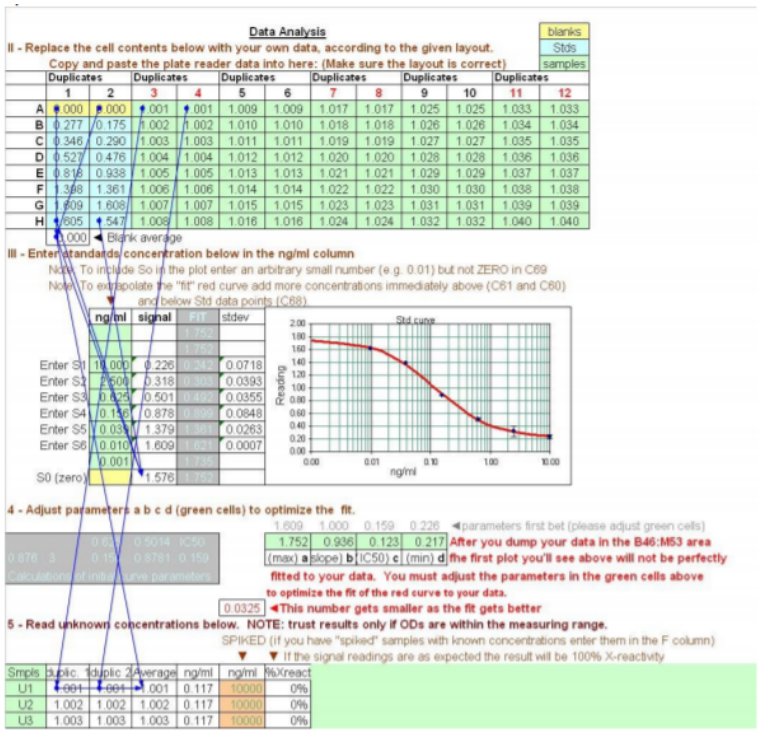
1. Into each well of the immunoplate add 25ul antiserum (in ELISA buffer). Add 25ul ELISA buffer to blank wells.
2. Incubate at room temperature for 1 hour.
3. Add 50ul standard or sample (in diluent). Do not wash plate before adding. Add 50ul diluent to blank wells.
4. Incubate at room temperature for 2 hours. Shorter pre-incubations may result in lower sensitivity.
5. Rehydrate the Bt-tracer (in ELISA buffer) and add 25ul per well.
6. Incubate at 4°C overnight. For best results re-equilibrate to RT before proceeding.
7. Wash immunoplate 5 times with 300ul per well of ELISA 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 300ul of ELISA buffer into each well and gently shake for at least a few seconds. Thorough washing is essential.
8. Add 100ul per well of streptavidin-HRP. Tap or centrifuge the SA-HRP vial to collect all liquid contents on the bottom of the vial. Dilute 1/200 in EIA buffer (e.g. 60ul in 12ml) and vortex gently. Add 100ul to all wells, including the blanks.
9. Incubate at room temperature for 1 hour.
10. Prepare TMB chromogenic solution immediately before use by mixing 20 parts of the TMB substrate buffer (citrate, brought to room temperature) with 1 part TMB -H_2O_2 solution (TMB substrate stock). This dilution should be used within 15 minutes after preparation.
11. Wash immunoplate 5 times (see step 7).
12. Add 100ul per well of TMB solution from step 10. Add to all wells, including the blanks.
13. Incubate at room temperature (usually 10 - 30 minutes). You may read the developing blue color at 650nm and use the data for your calculations.
14. Terminate reactions by adding 100ul 2N HCl per well.

15. Read absorbance at 450nm within fifteen minutes.

Calculation

We recommend that you use curve fitting software for your data analysis. Plate readers often include such software packages. This is, however, not essential and you may opt to plot manually on semi-log paper. You can also use a spreadsheet program. Should you need help with the latter method we recommend the following procedure.

Set up a spreadsheet as shown below (note that the values on the spreadsheet are merely illustrative and are not necessarily typical for this particular kit).



Set up an 8 x 12 area to match the layout of the plate and copy the plate reader data in it.

Calculate the average of the blanks on another cell as indicated by the arrows starting from cells A1 and A2.

Enter the concentration of the standards (see under ng/ml in figure).

Calculate the average of the ODs of the standards and subtract the background (blank) as indicated by the arrows for the last standard (cells H1 and H2).

Make a standard curve by plotting the OD readings (minus the blank average) against the standard concentrations in ng/ml.

Use the equation shown below to calculate the values on the "FIT" column and plot a smooth line of FIT values versus standard concentrations. Then change the parameters a (max), b (slope), c (IC50), and d (min), until you are satisfied that fit is good.

$y = [(a-d) / (1 + (x/c)^b)] + d$

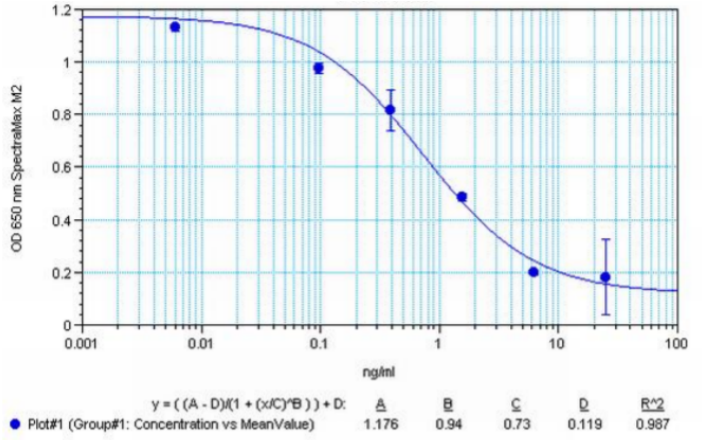
Next, calculate the average of your sample readings and subtract the blank average (see arrows starting from A3 and A4, and the arrows leading to "Average").

Finally, you may isolate x in the equation above to calculate the concentrations in ng/ml for all your samples:

$x = c ((y-a) / (d-y))^{1/b}$

Caution: when you calculate sample concentrations using the "reverse" equation if y = d or y> a or y < d, the reading is out of range and the calculation will yield an error or a meaningless negative concentration.

Typical Standard Curve



Detection Range

0-25ng/ml

Specificity

Exendin-4, 100%

Exendin-3, 100%

Exendin-4 (3-39), 100%

Exendin-4 (9-39), 100%

GLP-1 (7-36) amide (human, rat, mouse, bovine, guinea pig), 0%

GLP-1 (7-37) (human, rat, mouse, bovine, guinea pig), 0%

GLP-1 (1-37) (human, rat, mouse, bovine, guinea pig), 0%

GLP-2 (1-34) (human), 0%

Glucagon (1-29) (human), 0%