Version 04-12/24

User's Manual

Deslorelin High Sensitivity ELISA Kit

REF **DEIA-XYZ69**

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

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

Formulated for extracted samples.

General Description

Deslorelin is a 9-amino acid synthetic peptide analogue of the natural gonadotrophin releasing hormone (GnRH). It binds with high affinity to the GnRH receptor on anterior pituitary cells, where it acts as an agonist. Deslorelin stimulates the secretion of LH and FSH from the anterior pituitary and it has potential therapeutic applications in the treatment of endometriosis, uterine leiomyoma (fibroids) as well as malignant neoplasms. Recently, Deslorelin has been used in veterinary medicine for various indications.

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.

The sequence of the standard peptide is shown on the datasheet (note that large protein sequences are usually not shown).

The standard is used to make a standard curve in the range specified in the kit's datasheet. Standard curves are S-shaped (on a semi-log plot) but for a few kits they appear to be almost linear over the kit's range. The measuring range is the range of standard concentrations near the middle or near the IC050 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. ELISA buffer concentrate (50 ml 20x concentrate)
2. 96-well immunoplate with acetate plate sealer
3. Anti-serum (lyophilized powder)
4. Standard (1 µg target peptide)
5. Biotinylated tracer (lyophilized powder)
6. Sreptavidin-HRP (100 µl 200x concentrate)
7. TMB substrate solution 1.5 ml
8. TMB-H2O2 stock solution 1.5 ml
9. Stop solution 2 N HCl 15 ml

10. Datasheet

Materials Required But Not Supplied

1. 96-well microtiter plate reader set up to measure 450 nm and 650 nm
2. 96-well plate washer and shaker (optional)
3. Distilled or deionized water, or comparable quality
4. Curve fitting software (optional, use free online services)
5. Test tubes, pipettes and various other standard laboratory items
6. Sample Extraction Kit

Storage

After you receive the kit, store it in the refrigerator (4 - 8°C) for 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 removing them by filtration prior to usage.

Specimen Collection And Preparation

1. **Sample extraction**. Sample extraction is recommended for serum or plasma samples used in high sensitivity absorbance assays. It may be less important for tissue culture samples. See "Suggested Protocol for Sample Extraction" below for details. The kit may still be used without extraction but this may cause unexpected results due to the possible binding between serum proteins and kit components.
2. **Sample concentration**. The concentration of the target molecule must be within the measuring range of the kit (most precise results will be achieved in the linear part of the standard curve around the IC50). 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.

SUGGESTED PROTOCOL FOR SAMPLE EXTRACTION

You can use standard to determine if extraction is required. For example, if you are working with serum, you may spike it with known amounts of standard and check if they are accurately determined by the assay with and without extraction. Extraction eliminates potentially interfering substances, such as albumin.

Extraction may also be necessary to concentrate the sample to within the measuring range. As with any purification technique, recovery of the desired substance is likely to be incomplete. Therefore, both optimization and quantification of the extraction procedure are recommended for more accurate determinations. While we cannot provide you with extraction optimization and quantification protocols, we have included enough standard in the kit should you wish to use it for this purpose

C18 Sep-Column Extraction Method

The following generic protocol is meant to help users with extracting their samples. It should be applicable to different biological fluids but should not be thought of as an optimized protocol for any particular antigen.

Required Materials (must be purchased seperately)

SEP-Column containing 200 mg of C18

Buffer A: 1% trifluoroacetic acid (TFA, HPLC Grade). (Acidifies plasma sample to remove interfering proteins such as albumin)

Buffer B: 60% acetonitrile (HPLC Grade), 1% TFA, and 39% distilled water. (Elutes peptide from column) You may also consider purchasing Extraction kits (Contact CD for Cat. No.), which include SEPcolumns and buffers A & B for 50 samples.

Withdrawal and Preparation of Plasma

Collect blood samples (2 - 6 ml) into a chilled syringe and transfer into a polypropylene tube containing EDTA (1 mg/ml of blood) as an anticoagulant and Aprotinin (500 kIU/ml of blood) as a protease inhibitor at 4°C. Do not use heparinized tubes as they may interfere with the assay. Vacutainers with EDTA are acceptable. Centrifuge blood at 1,600 x g for 15 minutes at 4°C. Collect the top (plasma) layer. Proceed to extraction immediately or freeze at -70°C for later use.

Extraction Procedure

1. Add an equal amount of Buffer A to the plasma.
2. Centrifuge at 6,000 x g to 17,000 x g for 20 minutes at 4°C.
3. Transfer supernatant to a new tube discarding any pellet that may be present.
4. Equilibrate a SEP-Column by washing with 1 ml Buffer B followed by 3 x 3 ml Buffer A.
5. Load the plasma solution onto the equilibrated SEP-Column.
6. Slowly wash the column with Buffer A (3 ml, twice) and discard the wash. A light vacuum (10 sec/drop) may be applied to the column.
7. Elute the peptide slowly with Buffer B (3 ml, once) and collect eluant in a polypropylene tube. A light vacuum may be applied as in previous step.
8. Freeze-dry eluant to dryness using a dry ice/methanol bath to freeze the sample and a centrifugal concentrator to evaporate it.
9. Dissolve the residue in a suitable volume of ELISA buffer such that the concentration of the substance of interest will fall close to the IC50 (within the measuring range).

Reagent Preparation

Lyophilized kit components should not be re-hydrated until they are needed.

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 50 ml contained in the kit with 950 ml of water.

3. Standard: If samples are to be extracted and re-suspended. Add 1 ml of ELISA buffer to the vial of lyophilized standard peptide (1 µg) and mix gently.
4. Standard curve. Make serial dilutions of the standard to cover the range of this kit.
5. Antiserum. Add 5 ml of ELISA buffer and mix gently.
6. Biotinylated tracer. Add 5 ml of ELISA buffer to the vial of lyophilized biotinylated peptide and mix gently. Please check the datasheet for exceptions.

Assay Procedure

1. **ELISA buffer and Diluent** Antiserum and Bt-tracer are always reconstituted and used in ELISA buffer. The standards and samples are prepared in "standard diluent" (or 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 not influence concentration calculations - thus, they are optional.

LAYOUT

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

Protocol:

1. **Into each well of the immunoplate add**

   50 µl standard or sample (in ELISA buffer)

   25 µl antiserum (in ELISA buffer),

Add 75 µl ELISA buffer to blank wells.

2. **Incubate at room temperature for 1 hour.**
3. **Add 25 µl Bt-tracer (in ELISA buffer) per well including the blanks.**
4. **Incubate at room temperature for 2 hours.**
5. **Wash immunoplate 5 times with 300 µl 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 300 µl of ELISA 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.** Tap or centrifuge the SA-HRP vial to collect all liquid contents on the bottom of the vial. Dilute 1/200 in ELISA buffer (60 µl in 12 ml) and mix gently. Add 100 µl to all wells, including the blanks.
7. **Incubate at room temperature for 1 hour.**

8. **Prepare TMB chromogenic solution** immediately before use by mixing 20 parts of the Substrate buffer (citrate, brought to room temperature) with 1 part TMB - H2O2 Stock Solution. This dilution should be used within 15 minutes after preparation.
9. **Wash immunoplate 5 times (see step 3).**
10. **Add 100 µl per well of freshly prepared TMB chromogenic solution.** Add to all wells, including the blanks.
11. **Incubate at room temperature (usually 10 minutes).** This can be adapted according to how fast the color reaction takes place. You may read the developing blue color at 650 nm to decide when to stop the color reaction.
12. **Terminate reactions by adding 100 µl 2 N HCl per well.**
13. **Read absorbance at 450 nm within 15 minutes** (and optionally at 650 nm for background correction).

Quality Control

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 kit's standard.** Ideally the kit's synthetic standard mimics the natural peptide perfectly. Sometimes, however, natural peptides exist as families of species related by a common or similar sequence. Also, natural peptides may be modified enzymatically or spontaneously, 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.
4. **Sample extraction.** Factors present in serum can bind to EIAH kit components. The effects can vary from negligible to complete obliteration of signal. Therefore, sample extraction may be required prior to using the kit.

Calculation

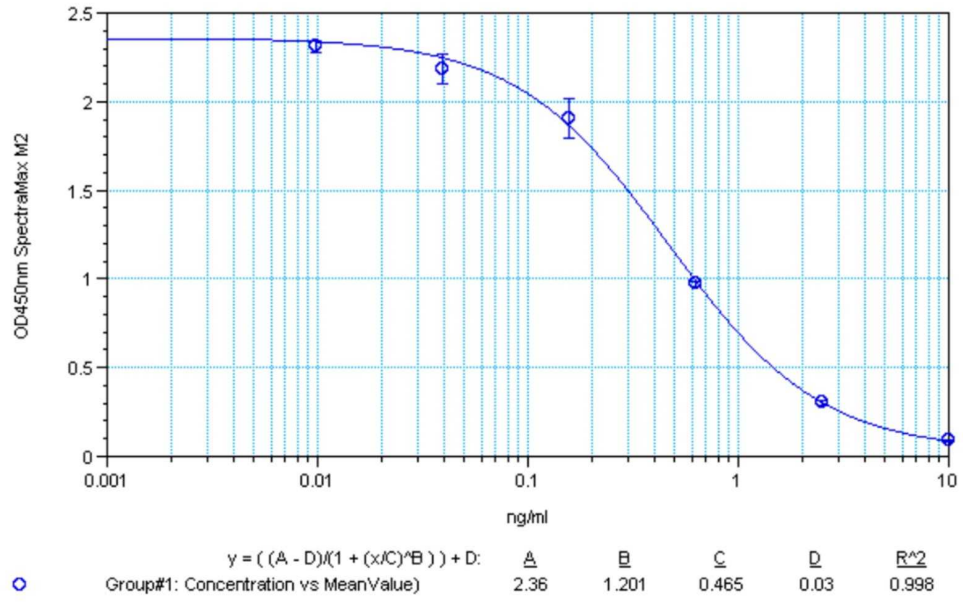
Plot data and calculate results. We recommend that you use curve fitting software for your data analysis. Plate readers often include such software packages, otherwise use free online tools that support 4 parameter logistic fitting (4PL) also called dose-response curve, i.e. https://mycurvefit.com/. Statistical software such as Origin™, Prism™ or R are also capable of such calculations. This is, however, not essential and you may opt to plot manually on semi-log paper or use a spreadsheet program.

If you e-mail us (contact information on front cover) we will be happy to send you a working Excel spreadsheet for fitting your curve within Excel.

Typical Standard Curve

Typical titration curve of Deslorelin in a competitive ELISA with this antibody:

Suggested Preparation of Standards		
	ng/ml	Range: 0.01 to 10ng/ml
Stock	1000	
S1	10.00	Add 10µl Stock + 990µl diluent
S2	2.50	Add 200µl S1 + 600µl diluent
S3	0.63	Add 200µl S2 + 600µl diluent
S4	0.16	Add 200µl S3 + 600µl diluent
S5	0.04	Add 200µl S4 + 600µl diluent
S6	0.01	Add 200µl S5 + 600µl diluent
S0	0.00	500µl diluent



Performance Characteristics

Average IC50: 0.4 ng/ml

Detection Range

0-10 ng/ml

Specificity

PEPTIDE: %:

(Des-Gly10, D-Trp6, Pro-NHEt9)-LHRH (Deslorelin) 100%

(Des-Gly10, D-Leu6, Pro-NHEt9)-LHRH (Leuprolide) 100%

LHRH 0%

LHRH (lamprey) 0%

LHRH (salmon) 0%

LHRH (chicken) 0%

[D-Ala6]-LH-RH 0%

[D-Phe2, Pro3, D-Phe6]-LH-RH 0%

[D-Phe1,2,D-Trp3,6]-LH-RH 0%

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