Version 28-04/25

User's Manual

Human Octreotide ELISA Kit

REF **DEIA-XYZ103**

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.

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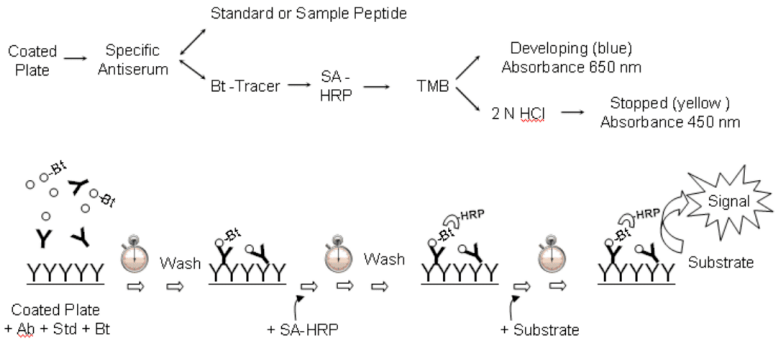
PRODUCT INFORMATION

Intended Use

This ELISA has been validated with the included reagents. It is intended to be used with samples of human origin. For research use only.

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 $IC0_{50}$ of the standard curve. Unknown sample concentrations are measured by comparing their absorbance with the standard curve.

We include sufficient reagents for 96 determinations.

Variation . Accuracy . Extraction . Cross-reactivity

The kit's IC_{50}, 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:

Both samples and standards must be measured in the same diluent and under the same conditions (same microtiter plate). 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.

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 altemative 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.

Reagents And Materials Provided

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.

1. ELISA Buffer Concentrate (50 ml 20× concentrate)
2. 96-well Immunoplate with Plate Sealer
3. Antiserum (lyophilized powder)
4. Standard (lyophilized powder with 1μg target peptide)
5. Biotinylated Tracer (lyophilized powder)
6. Streptavidin_HRP(100 μl 200× concentrate)
7. TMB-H_2O_2 Stock Solution (1.5 ml)
8. Substrate Buffer (15 ml)
9. Stop Solution 2 N HCl(15 ml)
10. Standard Diluent - Peptide-free human Serum (9 ml)

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

Storage

One year from production date. Store refrigerated at 4° - 8°C.

Specimen Collection And Preparation

1. Sample extraction. EIAS - extraction-free absorbance assays can be used with human serum or plasma (according to its designation) without performing an extraction.
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.

PREPARE KIT COMPONENTS

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 50 ml contained in the kit with 950 ml of water.
3. Standard diluent. Depending on the type of kit (check datasheet) the standards and samples are diluted in the following diluent:

   - EIAS type ELISA kit: species-specific treated serum
4. Standard. Add 1 ml of standard diluent to the vial of lyophilized standard peptide (1µg) and mix 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.
5. Standard curve. Make serial dilutions of the standard to cover the range of this kit. Please check the included datasheet for the appropriate range and dilution suggestions.
6. Antiserum. Add 5 ml of ELISA buffer and mix gently.
7. Biotinylated tracer. Add 5 ml of ELISA buffer to the vial of lyophilized biotinylated peptide and mix gently. Please check the datasheet for exceptions.

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

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).

For EIAS (extraction-free) kits it is the species-specific treated serum provided with the kit.

If there is no interference with the kit's components, you should use your own diluent for your samples and standards. However, the standard curve should show similar characteristics as the one from the datasheet Room Temperature. Reagents, samples, and the plate should be brought to room temperature before use.

2. Shakers. Shakers (optional) may help lower the experimental variation of duplicates (recommended at 60

rpm).

3. 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.

Assay Procedure

1. Into each well of the immunoplate add 25 µl antiserum (in ELISA buffer). Add 25 µl ELISA buffer to blank wells.
2. Incubate at room temperature for 1 hour.
3. Add 50 µl standard or sample (in serum). Do not wash plate before adding. Add 50 µl serum to blank wells.
4. Incubate at room temperature for 2 hours.
5. Add 25 µl Bt-Tracer (in ELISA buffer) per well including the blanks
6. Incubate at 4°C overnight. Shorter incubation times may result in low signal. For best results re-equilibrate to RT before proceeding.
7. 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 ul of ELiISA buffer into each well and gently shake for at least a few seconds. Thorough washing is essential.
8. 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 ul 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 Substrate buffer (citrate, brought to room temperature) with 1 part TMB - H_2O_2 Stock Solution. This dilution should be used within 15 minutes after preparation.
11. Wash immunoplate 5 times (see step 7).
12. Add 100 µl per well of freshly prepared TMB chromogenic solution. Add to all wells, including the blanks
13. 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.
14. Terminate reactions by adding 100 ul 2 N HCl per well.
15. Read absorbance at 450 nm within 15 minutes (and optionally at 650 nm for background correction).

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, PrismM 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.

Specificity

Octreotide: 100%

Somatostatin-14: 0%

Somatostatin-28: 0%

Somatostatin Analog,RC-160: 0%

Somatostatin Analog,(CTOP): 0%