Version 28-02/25

User's Manual

Exendin-4 (Heloderma suspectum) - ELISA Kit

REF DEIA-BJ815

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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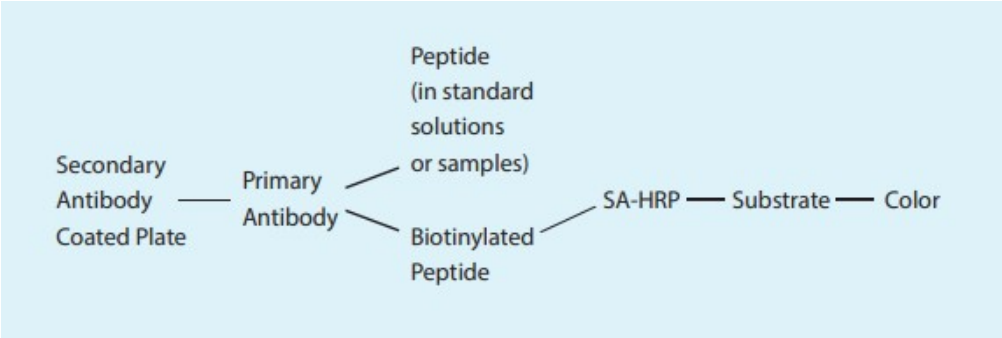
PRODUCT INFORMATION

Intended Use

This kit is designed to measure the concentration of a specifi c peptide and its related peptides based on the principle of a "competitive" enzyme immunoassay.

Principles of Testing

The immunoplate in this kit is pre-coated with a secondary antibody, whose nonspecifi c binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody. This primary antibody's Fab fragment will then be competitively bound by both the biotinylated peptide and the targeted peptide in either the standard peptide solution or the unknown sample. The biotinylated peptide interacts with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution. The intensity of the resulting yellow color is directly proportional to the amount of biotinylated peptide-SA-HRP complex, but inversely proportional to the amount of the targeted peptide (in either the standard peptide solution or the unknown sample). This is due to competition between the biotinylated peptide and the target peptide for binding with the primary antibody. A standard curve can be established by plotting the measured O.D. as a function of the various known standard peptide concentrations. Unknown peptide concentration in samples can then be determined via extrapolation based on this standard curve.



Reagents And Materials Provided

1. EIA assay buffer concentrate (20x, 50ml)
2. Pre-coated EIA plate (96 wells, 1 plate)
3. Acetate plate sealer (APS) (3 pieces)
4. Primary antibody (1 vial)
5. Standard peptide (1 vial)
6. Biotinylated peptide (1 vial)
7. Positive control (2 vials)
8. Streptavidin-horseradish peroxidase (SA-HRP) concentrate (30µl)
9. Substrate solution (12ml)
10. 2N HCl (Stop solution) (15ml)

Materials Required But Not Supplied

1. Microtiter plate reader (450nm) (required)
2. Micropipette with disposable pipette tips (required)
3. Absorbent material for blotting (required)
4. Vortex (required)
5. Curve-fi tting software capable of 4 parameter logistics (recommended)
6. Orbital plate shaker (300-400rpm) (recommended)
7. Microtiter plate washer (recommended)
8. Multi-channel pipette (50-100µl) (recommended)
9. Solution reservoir (recommended)
10. Centrifuge (optional)
11. Blood collection tubes (no EDTA) (optional)
12. Aprotinin (30 TIU) (optional)
13. C18 SEP-COLUMN (optional)
14. Buffer A (optional)
15. Buffer B (optional)

Storage

1. Store the kit at 4°C upon receipt. Do not freeze.
2. It is highly recommended that all solutions be used as soon as possible after reconstitution.
3. Any unused strips/columns may be removed from the pre-coated immunoplate. Please place strips back in the original zip-lock foil pouch with a dessicant, reseal, and store at 4°C. Do not allow moisture to accumulate on the wells.
4. If necessary, store the 1× assay buffer at 4°C.
5. If necessary, store any reconstituted solutions of standard peptide, biotinylated peptide, antibody and SA-HRP at 4°C

Specimen Collection And Preparation

General Blood Withdrawal and Plasma Collection:

1. Collect blood samples into Vacutainer tubes, which can hold up to 7ml of blood.

   Note: To reduce background noise with this Exendin-4 EIA kit, avoid adding EDTA to samples.
2. Gently rock the Vacutainer tubes several times immediately after collection of blood to avoid coagulation.
3. Transfer the blood to centrifuge tubes containing aprotinin (Catalog no. RK-APRO), and gently rock several more times to inhibit the activity of proteases.

   Note: We recommend 0.6 TIU, or 100µl, of Aprotinin per 1ml of blood collected. If the Vacutainer tubes are centrifuge-safe, the Aprotinin may be added directly to them.

4. Centrifuge the blood at 1,600 ×g for 15 minutes at 4°C and collect the plasma. Note: Plasma can be kept at -70°C and will remain stable for up to one month.
5. For peptide extraction from sample, acidify the plasma with an equal amount of Buffer A. Mix and centrifuge at 6,000 to 17,000 ×g for 20 minutes at 4°C. This will be loaded onto the C-18 SEP-COLUMN.

   Note: We recommend using at least 1ml of plasma for peptide extraction. It may be possible to perform the extraction using smaller volumes as long as volumes of reconstituting and eluting buffer are adjusted accordingly.

Extraction of Peptides from Sample:

1. Equilibrate a SEP-COLUMN containing 200mg of C18. Wash with 1ml of Buffer B once, followed by 3ml of Buffer A three (3) times.
2. Load the acidified sample (plasma, serum, tissue, etc.) solution onto the pre-equilibrated C-18 SEP-COLUMN.
3. Slowly wash the column with 3ml of Buffer A twice and discard the wash.
4. Elute the peptide slowly with 3ml of Buffer B once and collect the eluant into a polystyrene tube.

   Note: Ensure there is a constant flow for all solutions during the extraction procedure. For optimal sample processing and recovery, do not allow air bubbles to enter the C-18 matrix.
5. Evaporate eluant to dryness in a centrifugal concentrator or by a suitable substitute method.

   Note: A combination of a centrifugal concentrator (i.e. Speedvac) and a lyophilizer (freeze-dryer) produces the best results. First, use a centrifugal concentrator to dry the sample for approximately 15 minutes, removing the organic layer. Snap-freeze the remaining sample and freeze-dry overnight using a lyophilizer. If a centrifugal concentrator is not accessible, freeze-drying overnight using a lyophilizer will be sufficient.
6. Keep the dried extract at -20°C and perform the assay as soon as possible. Use the 1× assay buffer to reconstitute the dried extract to the desired concentration. If the peptide value does not fall within the range of detection, dilute or concentrate the sample accordingly.

   Note: For example, if 1ml of plasma was extracted, dried, and then reconstituted in 250µl of 1× assay buffer, then the original sample would have now have undergone a 4× concentration.

Assay Procedure

Note:

1. Read this protocol in its entirety before starting the assay. Each kit contains reagents suffi cient for 96 wells and is capable of assaying 40 duplicate samples.
2. The kit and all its components should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. Before opening any Eppendorf tubes for reconstitution, briefly centrifuge at ~3,000rpm for 5 seconds to ensure that all the lypholized material is at the bottom of the tube.

Procedure

1. Dilute the 20× EIA assay buffer concentrate with 950ml of distilled water. Mix thoroughly before use. This will be the 1× assay buffer solution used to dilute or reconstitute all other samples and reagents during the assay.

   Note: If crystals appear in the 20× assay buffer, the bottle can be placed in a warm water bath for

approximately 30 minutes or until no crystals are visible.

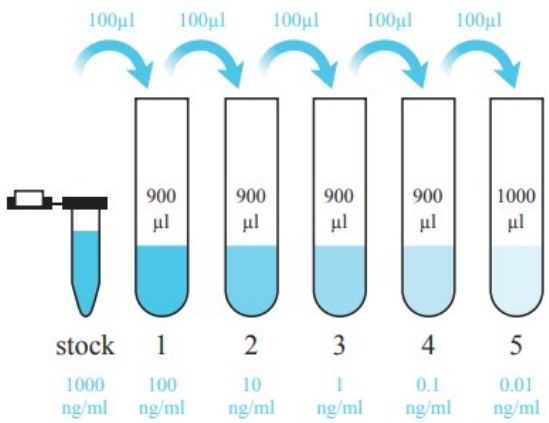
2. Reconstitute the standard peptide in 1ml of the 1× assay buffer and vortex thoroughly. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. This will be the standard stock solution.

   Note: Vortex immediately before use.

3. Reconstitute the primary antibody in 5ml of 1× assay buffer and vortex thoroughly. Allow the solution to sit for at least 5 minutes at room temperature to completely dissolve.
4. Reconstitute the biotinylated peptide in 5ml of 1× assay buffer and vortex thoroughly. Allow the solution to sit for at least 5 minutes at room temperature to completely dissolve.
5. Reconstitute the positive control in 200µl of 1× assay buffer and vortex thoroughly. Allow the solution to sit for at least 5 minutes at room temperature to completely dissolve.
6. Prepare the standard dilutions from the rehydrated standard peptide. Vortex the tube thoroughly after each serial dilution.

   Prepare peptide standard solutions as follows:

Standard ID / Number	1x Assay Buffer Volume	Standard Peptide Volume	Concentration
Stock	1000µl	(powder)	1000ng/ml
#1	900µl	100µl of stock	100ng/ml
#2	900µl	100µl of #1	10ng/ml
#3	900µl	100µl of #2	1ng/ml
#4	900µl	100µl of #3	0.1ng/ml
#5	900µl	100µl of #4	0.01ng/ml

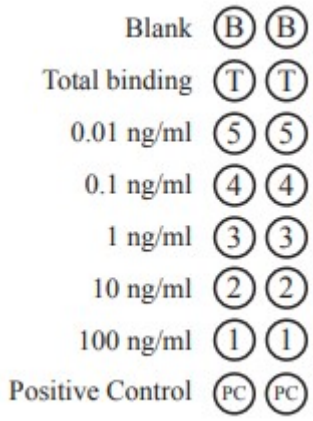


7. Leave wells A1 and A2 on the immunoplate empty as blanks.
8. Add 50µl of 1× assay buffer into wells B1 and B2. These will represent total binding (of the biotinylated peptide).
9. Add 50µl of the least concentrated peptide standard solution (#5) to wells C1 and C2. Next, add peptide standard #4 into wells D1 and D2, and so forth, continuing in the opposite order of the standard dilution.

   Note: Standard peptides should always be assayed in duplicate.

10. Add 50µl of rehydrated positive control into wells H-1 and H-2.

Note: Positive controls should always be assayed in duplicate.



11. Add 50µl of any unknown/prepared samples into their designated wells, again in duplicate.

    Note: Each laboratory must determine the appropriate dilution factors and preparation for their samples to ensure that peptide levels are detectable and within the linear range of the standard curve.

12. Add 25µl of rehydrated primary antibody into each well except the blank wells (A1 and A2).

    Note: A multi-channel pipette is NOT recommended to load the primary antibody.

13. Add 25µl of rehydrated biotinylated peptide into each well except the blank wells (A1 and A2).

    Note: A multi-channel pipette is NOT recommended to load the biotinylated peptide.

14. Seal the immunoplate with an acetate plate sealer (APS). Incubate the immunoplate for 2 hours at room temperature (20-23°C).

    Note: Orbital shaking at 300-400 rpm is recommended for the duration of the incubation.

15. Centrifuge the SA-HRP vial (3,000-5,000 rpm for 5 seconds) to mix. Pipette 12µl of SA-HRP into 12ml of 1× assay buffer and vortex the solution thoroughly.
16. Remove the APS from the immunoplate and discard the contents of the wells. Wash each well with 350µl of 1× assay buffer, discard the buffer, invert the immunoplate, and blot the plate dry. Repeat 3 more times.
17. Add 100µl of SA-HRP solution into each well.
18. Reseal the immunoplate with an APS. Incubate for 1 hour at room temperature (20-23°C).

    Note: Orbital shaking at 300-400 rpm is recommended for the duration of the incubation.

19. Remove the APS from the immunoplate and discard the contents of the wells. Wash each well with 350µl of 1× assay buffer, discard the buffer, invert the immunoplate, and blot the plate dry. Repeat 3 more times.
20. Add 100µl of the TMB substrate solution into each well.

    Note: TMB is light-sensitive. After the addition of the TMB substrate solution, it is strongly recommended to cover the immunoplate to protect it from light.

21. Reseal the immunoplate with an APS. Incubate for 1 hour at room temperature (20-23°C).

    Note: Orbital shaking at 300-400 rpm is recommended for the duration of the incubation.

22. Remove the APS from the immunoplate. Do NOT wash or the immunoplate or discard the contents of the wells.
23. Add 100µl of 2N HCl into each well to stop the reaction. The color in the wells should change from blue to

yellow. Gently tap the plate to ensure thorough mixing.

Note: Proceed to the next step within 20 minutes.

24. Load the immunoplate onto a microtiter plate reader and measure absorbance O.D. at 450nm.

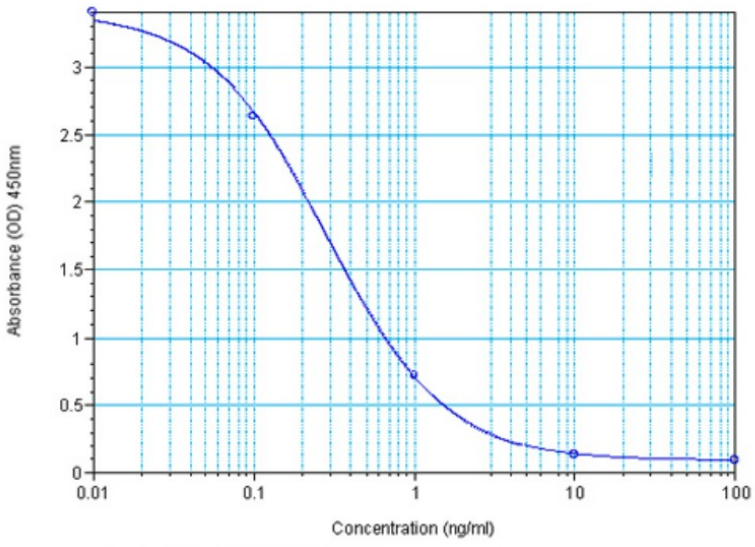
Calculation

1. Label the X-axis (log scale) with the concentration of standards #5 through #1 (0.01 to 100 ng/ml).
2. Label the Y-axis (linear scale) as absorbance (O.D.) at 450nm.
3. Average all duplicate readings (standards, positive control, samples) and substract the average blank O.D. reading.
4. Plot the O.D. for each standard peptide concentration directly above its X-axis coordinate.
5. Draw the best fi t curve through these data points. It should show a direct relationship between peptide concentration and absorbance. As the standard peptide concentration increases, the yellow color decreases, thereby reducing absorbance (O.D.).

   Note: We strongly recommend using curve-fi tting software capable of 4 parameter logistics or log-logit functionality.
6. To determine the peptide concentration in any unknown samples, fi rst locate its absorbance (O.D.) on the Y-axis. Draw a horizontal line across the graph from that absorbance to the intersection with the standard curve. The X-axis coordinate at this intersection point will correspond to the peptide concentration (ng/ml) in the assayed sample.

   Note: Multiply the measured peptide concentration by any dilution factor(s) used while preparing the original sample.
7. Refer to the QC Data Sheet for acceptable values of the positive controls.

Typical Standard Curve



Precision

Intra-assay variation: <10%

Inter-assay variation: <15%

Detection Range

0.08 - 0.86 ng/ml

Sensitivity

0.08 ng/ml

Specificity

Peptide	*%*
Exendin-4 (Heloderma Suspectum)	100
Exendin-3 (9-39)-NH2	100
Exendin-4 (3-39)	100
Lixisenatide	100
Glucagon (Human, Rat, Mouse, Porcine, Bovine)	0
GLP-1 (7-36)-NH2 (Human, Rat, Mouse)	0
GLP-1 (7-37) (Human, Rat, Mouse)	0
GLP-2 (Human)	0
Oxyntomodulin (Human, Rat, Mouse)	0

Precautions

1. Reagents of different lot numbers should never be mixed.
2. Plasma, serum, culture media, tissue homogenate, CSF, urine, or any biological fl uid can be assayed as long as the level of peptide in the sample is high enough for the sensitivity of the specific kit.
3. High levels of interfering proteins may cause variations within sample results. Therefore, it is imperative to select the appropriate sample preparation procedure to obtain optimal results. Please consult the literature for specific methodology.
4. When handling the plate, avoid touching the bottom. Any fi ngerprints or blots may affect the O.D. readings.
5. Manual washing may cause high duplicate coeffi cient variations. To reduce this factor, liquid from the plate should be removed by inverting and blotting the plate on an absorbent material.
6. Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into its container a few times to wet the pipette walls prior to loading the pipette.
7. Avoid submerging the whole pipette tip into reagents and samples. Droplets can accumulate at the end of the tip, causing an excess of solution to be loaded into the well and affecting assay results.
8. Performing this procedure outside of the recommended room temperature (20-23°C) may affect assay results.

9. Any modifi cations to the existing protocol (i.e. standard dilutions, pipetting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the sensitivity, specifi cty and results of the assay.

References

1. Porstmann, T. and Kiessig, S.T., Enzyme Immunoassay Techniques, An Overview, Journal of Immunological Methods, 150: 5-21 (1992).
2. Avrameas, S., Amplifi cation Systems in Immunoenzymatic Techniques, Journal of Immunological Methods, 150: 23-32 (1992).