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Leu-Enkephalin ELISA

Catalog Number M056014

For informational use only.
Please refer to insert included with product.

For the quantitative determination of Leucine-Enkephalin in human serum, plasma, urine, cell culture supernate, tissue homogenate, and CSF samples.

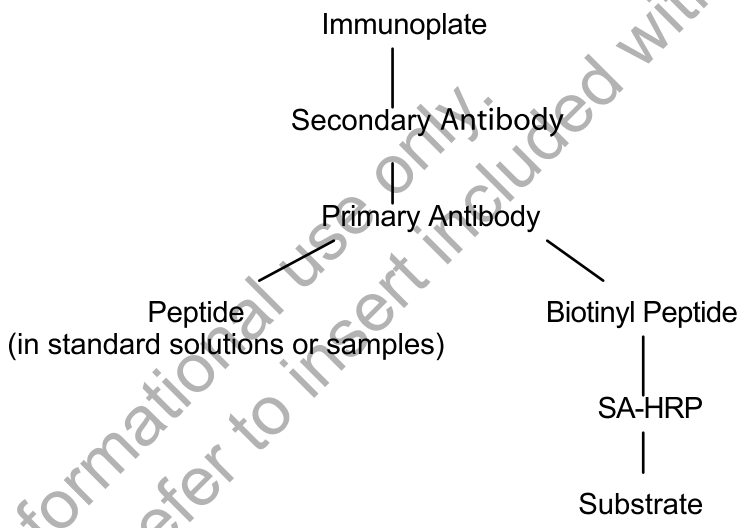
For research use only.
This product insert must be read in its entirety before using this product.

INTENDED USE

This Enzyme Immunoassay kit is designed to detect a specific peptide and its related peptides based on the principle of "competitive" enzyme immunoassay.

PRINCIPLE OF THE ASSAY

The immunoplate in this kit is pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample. The biotinylated peptide is able to interact with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution composed of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide to produce a blue colored solution. The enzyme-substrate reaction is stopped by hydrogen chloride (HCl) and the solution turns to yellow. The intensity of the yellow is directly proportional to the amount of biotinylated peptide-SAHRP complex but inversely proportional to the amount of the peptide in standard solutions or samples. This is due to the competitive binding of the biotinylated peptide and the peptide in standard solutions or samples to the peptide antibody (primary antibody). A standard curve of a peptide with known concentration can be established accordingly. The peptide with unknown concentration in samples can be determined by extrapolation to this standard curve.



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KIT COMPONENTS

Microtiter Plate - The plate contains 12 x 8-well strips. Ready for use.

Leu-Enkephalin Standard - 1 vial of human Leu-Enkephalin. Lyophilized.

Positive Control - human Leu-Enkephalin. Lyophilized. See vial for concentration.

Biotinylated Peptide - 1 vial of biotinylated Leu-Enkelphalin peptide. Lyophilized.

Rabbit Anti-Leu-Enkephalin Serum - 1 vial of a rabbit anti-Leu-Enkephalin IgG in serum. Lyophilized.

Streptavidin-HRP Concentrate - 1 vial of a concentrated solution of streptavidin-horseradish peroxidase.

Substrate Solution - 1 vial of a TMB solution.

Assay Buffer Concentrate - 1 vial of a 20-fold concentrated solution.

2N HCl - 1 vial of 2N HCl. Ready to use.

Plate Sealers - 3 adhesive strips.

STORAGE

Unopened Kit	Store at 2 - 8º C. Do not use past the kit expiration date.	
Opened Reagents	Biotinylated Peptide	Reagents must be used the same day when reconsituted.
	Rabbit Anti-Leu-Enkephalin Serum	
	Standard	
	Positive Control	
	Substrate	Store at 2 - 8 ºC.
	Streptavidin-HRP	
	2N HCl	
	Assay Buffer	
	Microtiter wells	Return unused wells to the foil pouch containing the desiccant and seal. Store at 2 - 8 ºC.

SUPPLIES REQUIRED BUT NOT PROVIDED

- Microplate Reader
- Microplate Washer
- Pipettes or pipetting equipment with disposable polypropylene tips
- Multi-channel pipette
- Disposable polypropylene test tubes
- Glass measuring cylinders
- Distilled or deionised water
- Extraction reagents/materials

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PRECAUTIONS

- 2N HCl is an acidic solution. Avoid contact with skin and clothing!
- Patient samples should be considered potentially contagious and be treated with the necessary safety precautions.
- An exchange of reagents between kits of different lot numbers is not possible.

CRITICAL PARAMETERS

- Allow samples and all reagents to equilibrate to room temperature (22–25 °C) prior to performing the assay. This is especially important for the TMB Substrate.
- Adhere to recommended incubation temperatures in the protocol as variations may cause inconsistent or poor assay results.
- It is essential that all wells are washed thoroughly and uniformly. When washing is done by hand, use a squeeze bottle and ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the plate sealer except when adding reagents and during reading.

- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

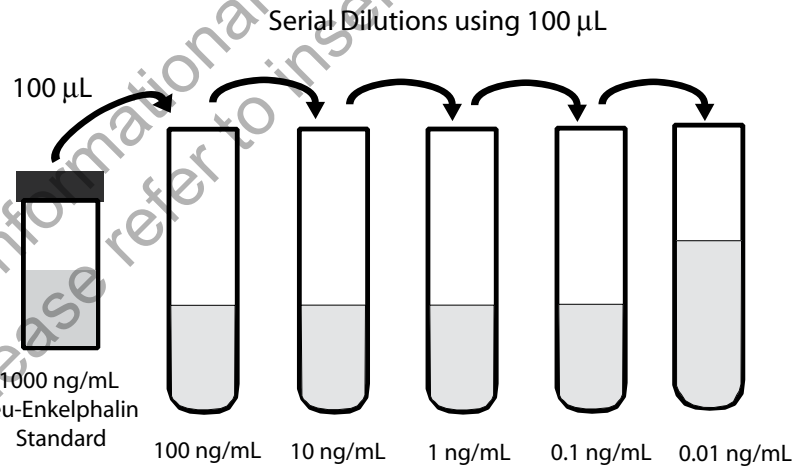
REAGENT PREPARATION

Note: All reagents should be stored at the recommended temperatures. Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.

Assay Buffer Concentrate - If precipitate is present, bring to room temperature and mix thoroughly before using. Dilute 50 mL of Assay Buffer Concentrate with 950 mL of deionized or distilled water to make Assay Buffer (1X). The Assay Buffer (1X) will be used to reconstitute all other reagents and for extraction of plasma samples.

Leu-Enkephalin Standard - Reconstitute standard peptide with 1 mL assay buffer, vortex. The concentration of this stock solution is 1,000 ng/mL. Label 5 polyproylene tubes 100, 10, 1, 0.1, and 0.01 ng/mL.

1. Pipette 900 µL of Assay Buffer (1X) into each tube.
2. Add 100 µL of the 1000 ng/mL stock into the 100 ng/mL tube and mix.
3. Pipette 100 µL from the 100 ng/mL tube into the 10 ng/mL tube. Mix.
4. Repeat the serial dilutions with the remaining tubes. The 100 ng/mL tube serves as the high standard. Assay Buffer (1X) serves as the 0 ng/mL standard.



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Positive Control - Reconsitute positive control with 200 µL assay buffer and mix thoroughly.

Rabbit Anti-Leu-Enkephalin Serum - Reconstitute primary anti-serum with 5 mL of Assay Buffer (1X) and mix thoroughly.

Biotinylated Peptide - Reconstitute biotinylated peptide with 5 mL of Assay Buffer (1X) and mix thoroughly.

Streptavidin-HRP Concentrate - Centrifuge the SA-HRP vial (500-1,000 r.p.m., 15 seconds, 4 °C) and pipette 12 µL SA-HRP into 12 mL Assay Buffer (1X) to make SA-HRP solution, vortex.

SAMPLE COLLECTION AND STORAGE

Collect blood samples into the Lavender Vacutaner tubes which contain EDTA and can collect 7 mL blood/ tube. Gently rock the Lavender Vacutaner tubes several times immediately after collection of blood for anti-coagulation. Transfer the blood from the Lavender Vacutaner tubes to centrifuge tubes containing aprotinin (0.6 TIU/mL of blood) and gently rock for several times to inhibit the activity of proteinases. Centrifuge the blood at 1,600 x g for 15 minutes at 4 °C and collect the plasma. Plasma kept at -70 °C may be stable for one month.

PLASMA EXTRACTION

Note: Extraction for plasma samples is recommended. A suggested extraction procedure has been included. All reagents and materials for extraction are not included.

Elution Solvents:

1. Buffer A : 1% trifluoroacetic acid (TFA, HPLC Grade) in H_2O.

2. Buffer B : 60% acetonitrile (HPLC Grade) in 1% TFA.

Extraction of Peptide from Plasma:

1. Acidify the plasma with an equal amount of buffer A. For example, if you are using 1 mL of plasma, add 1 mL of buffer A. Mix and centrifuge at 6,000 to 17,000 x g for 20 minutes at 4 °C.

2. Equilibrate a SEP-COLUMN containing 200 mg of C-18 by washing with buffer B (1 mL, once) followed by buffer A (3 mL, 3 times).

NOTE: From steps 3-5, no pressure should be applied to the column.

3. Load the acidified plasma solution onto the pre-treated C-18 SEP- COLUMN.

4. Slowly wash the column with buffer A (3 mL, twice) and discard the wash.

5. Elute the peptide slowly with buffer B (3 mL, once) and collect eluant in a polypropylene tube.

6. Evaporate eluant to dryness in a centrifugal concentrator or by a suitable substitute method.

7. Keep the dried extract at -20 °C and perform assay as early as possible. Reconstitute the dried extract with Assay Buffer (1X) before performing assay. If the peptide value exceeds or does not fall in the range of detection, dilute or concentrate the samples as needed.

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ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use. *Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding*.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microtiter strips from the plate frame and return them to the foil pouch containing the desiccant pack.

Standard/Sample Incubation

3. Leave well A-1 and A-2 empty as a Blank.
4. Add 50 µL of Assay Buffer (1X) into well B-1 and B-2 as Total Binding.
5. Add 50 µL of standards, controls or samples into remaining wells in duplicate.
6. Add 25 µL of anti-serum into each well except the Blank well.
7. Add 25 µL of Biotinylated Peptide into each well except the Blank well.
8. Cover the plate with the plate sealer provided. Incubate for 2 hours at room temperature (22 - 25 °C).

Wash

9. Aspirate and wash each well, except the blank, four (4) times by adding 300 µL of Assay Buffer (1X). Blot dry by inverting the plate on an absorbent material.

SA-HRP Incubation

10. Add 100 µL of diluted SA-HRP solution into each well except the Blank.
11. Cover with a plate sealer and incubate for 1 hour at room temperature.

Wash

12. Aspirate and wash each well, except the blank, four (4) times by adding 300 µL of Assay Buffer (1X). Blot dry by inverting the plate on an absorbent material.

Substrate incubation

13. Add 100 µL of TMB Substrate into each well including the blank well.
14. Cover the plate with the plate sealer and incubate for 1 hour at room temperature.

Stop Reaction

15. Add 100 µL of 2N HCl into each well (including Blank). Proceed to next step within 20 minutes.
16. Clean the bottom of the plate with 70% ethanol. Read absorbance at 450 nm.

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SUMMARY

Prepare reagents and samples as previously described.

Leave well A-1 and A-2 empty as Blank. Add 50 µL Assay Buffer (1X) into B-1 and B-2 as Total Binding.

Add 50 µL Standard, Control or sample in duplicate into remaining wells.

Add 25 µL of primary anti-serum into each well, except the Blank

Add 25 µL of Biotinylated Peptide into each well except the Blank.
Cover and Incubate for 2 hrs at RT (22 - 25 °C).

Wash each well (except Blank) 4 times with 300 µL Assay Buffer (1X). Blot dry.

Add 100 µL SA-HRP solution to each well except the Blank.
Cover and Incubate for 1 hr at RT.

Wash each well (except Blank) 4 times with 300 µL Assay Buffer (1X). Blot dry.

Add 100 µL Substrate Solution to each well including Blank well.
Cover and incubate for 1 hr at RT.

Add 100 µL 2N HCl to each well including Blank well.
Read Absorbance at 450 nm.

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CALCULATION OF RESULTS

Plot the standard curve on semi-log graph paper. Known concentrations of standard peptide and its corresponding O.D. reading is plotted on the log scale (x-axis) and the linear scale (y-axis) respectively. The standard curve shows an inverse relationship between peptide concentrations and the corresponding O.D. absorbances. As the standard concentration increases, the intensity of the yellow color, and in turn the O.D. absorbance, decreases.

The concentration of peptide in a sample is determined by plotting the sample's O.D. on the y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point will intersect the x-axis at a coordinate corresponding to the peptide concentration in the unknown sample.

REFERENCES

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

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TROUBLESHOOTING

Problem	Recommendation
Low Absorbance	• Check reagents for proper storage. • Control expiration date. • Check preparation of reagents. • Control incubation times and temperature. • Check reader wavelength.
High Absorbance/high zero standard value	• Check preparation of reagents. • Control incubation times and temperature. • Equilibrate ELISA reagents to room temperature (22 - 25 °C). • Ensure that every well of the ELISA plate is completely filled and emptied at every wash step. • Check that plates are blotted on tissue paper after washing.
Flat cure/poor reproduciblity	• Check reagents for proper storage. • Control expiration date. • Check preparation of working standards. • Check incubation times and temperatures. • Use separate reservoirs for pipetting different solutions with multichanned pipettes. Always use new pipette tips. • Check pipette calibration. • Ensure efficient washing procedure.

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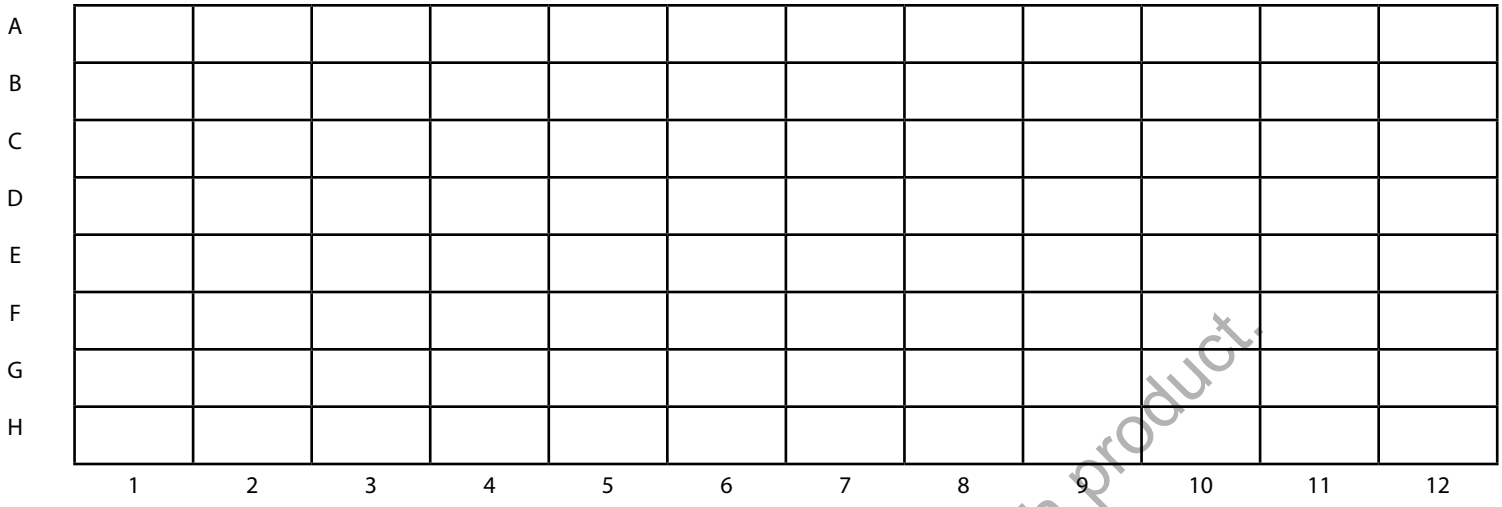
PLATE LAYOUT



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NOTES

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