BIOSCIENCES

β - Endorphin
***ELISA** [Enzyme-Linked ImmunoSorbent Assay]*

Specific quantitative assay for the determination of β-Endorphin in human plasma serum samples

For research laboratory use only.

Not for use in diagnostics procedures.

This Product is supplied by:

MD Biosciences, Division of Morwell Diagnostics GmbH
Gewerbestrasse 9, Postfach, 8132 Egg b. Zürich, Switzerland
Telephone (41-1) 986 26 28 Telefax (41-1) 986 26 30
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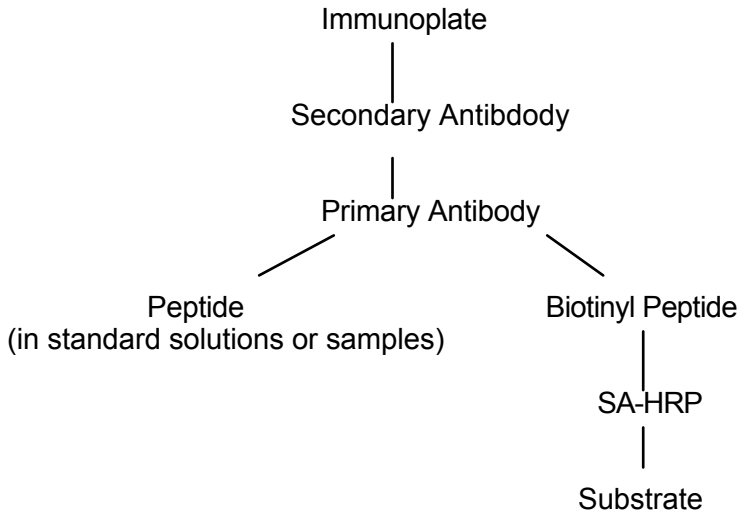
INTRODUCTION

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 ENZYME IMMUNOASSAY WITH THIS KIT

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-SA-HRP 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.



KIT MATERIALS

1. 20 x assay buffer concentrate (50 ml).
2. 96 well immunoplate (1 plate).
3. Acetate plate sealer (APS), (3 pieces).
4. Primary antiserum (rabbit anti-peptide IgG) (1 vial).
5. Standard peptide (1 ug).
6. Biotinylated peptide (1 vial).
7. Streptavidin-horseradish peroxidase (SA-HRP) (30 ul).
8. Substrate solution (12 ml).
9. 2N HCl (15 ml).
10. Assay diagram (1 sheet).
11. General protocol (insert).

NOTE:

Blood collection protocol and extraction procedure for plasma is provided for your information. (Materials for extraction not included).

ASSAY PROCEDURE

1. Carefully read this protocol before performing assay.
2. Dilute the assay buffer concentrate with 950 ml deionized or HPLC grade water. This assay buffer will be used to reconstitute all of the other compounds in this kit and the extract of plasma samples.
3. Rehydrate standard peptide with 1 ml assay buffer, vortex. The concentration of this stock solution is 1,000 ng/ml.
4. Prepare peptide standard solutions as follows:

Standard No.	Standard Volume	Assay Buffer	Concentration
Stock	1000 ml	----	1,000 ng/ml
#1	100 ul of stock	900 ul	100 ng/ml
#2	100 ul of #1	900 ul	10 ng/ml
#3	100 ul of #2	900 ul	1 ng/ml
#4	100 ul of #3	900 ul	0.1 ng/ml
#5	100 ul of #4	900 ul	0.01 ng/ml

5. Rehydrate primary antiserum with 5 ml of assay buffer, vortex .
6. Rehydrate biotinylated peptide with 5 ml of assay buffer, vortex.
7. Leave well A-1 empty as Blank.
8. Add 50 ul assay buffer into well B-1 as Total Binding.

9. Add 50 ul of the prepared peptide standard solutions from #5 to #1 (reverse order of serial dilution) into the wells from C-1 to G-1 respectively.

10. Add 50 ul samples into their designated wells.
11. Add 25 ul rehydrated primary antiserum into each well except the Blank well.
12. Add 25 ul rehydrated biotinylated peptide into each well except the Blank well.
13. Seal the immunoplate with acetate plate sealer (APS).
14. Incubate the immunoplate for 2 hours at room temperature.
15. Centrifuge the SA-HRP vial provided in this kit (500-1,000 r.p.m., 15 seconds, 4°C) and pipet 12 ul SA-HRP into 12 ml assay buffer to make SA-HRP solution, vortex.
16. Remove APS from the immunoplate
17. Discard contents of wells.
18. Wash each well (except the Blank) with 300 ul assay buffer, discard the buffer and blot dry the plate. Repeat 5 times.
19. Add 100 ul SA-HRP solution into each well except the Blank well.
20. Reseal the immunoplate with APS.
21. Incubate for 1 hour at room temperature.
22. Wash and blot dry the immunoplate 6 times with the assay buffer as described above.
23. Add 100 ul substrate solution provided in this kit into each well including the Blank well.
24. Reseal the immunoplate with APS.
25. Incubate for 1 hour at room temperature.
26. Add 100 ul 2N HCl into each well (including the Blank) to stop the reaction. Go to the next step within 20 minutes.
27. Clean the immunoplate bottom with 70% ethanol.
28. Remove APS and load the immunoplate onto a Microtiter Plate Reader.
29. Read absorbance O.D. at 450 nM. The O.D. reading for the TB (well B-1) is usually around 0.8-1.5.

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.

STORAGE

Store the kit at 2-4°C upon receipt. The kit will be stable for 6 months. The kit should be equilibrated to room temperature before assay.

EXTRACTION OF PEPTIDE FROM BLOOD PLASMA

- ***Blood Withdrawal***

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.

- ***Elution Solvents***

1. Buffer A : 1% trifluoroacetic acid (TFA, HPLC Grade) in H2O.
2. Buffer B : 60% acetonitrile (HPLC Grade) in 1% TFA.

- ***Extractionof 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 C18 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 before performing assay. If the peptide value exceeds or does not fall in the range of detection, dilute or concentrate the samples accordingly.

Specifications for β-Endorphin (Human)

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu

- Sensitivity: 0.18g/ml
- Precision: Intra-assay variation: <5%
  Inter-assay variation: <14%

- Range: 0-100ng/ml Linear Range: 0.18 - 2.51 ng/ml

- Specificity:

Peptide	Cross-reactivity
β-Endorphin (Human)	100
β-Endorphin (Rat)	100
Ac-β-Endorphin (Human)	100
Met-Enkelphalin (Human)	0
Leu-Enkelphalin (Human)	0

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*