beta-Endorphin RIA

Radioimmunoassay for the quantitative determination of β-Endorphin in human plasma.

REF MI11021

Σ 100

EU: For research use only.
U.S.: For research use only.
Not for use in diagnostic procedures.

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1. Principle of the Test

β-endorphin in sample (plasma, cerebrospinal fluid or tissue) is extracted using sep-pak C 18 cartridges. The extracts are analysed by a competitive radioimmunoassay using antibodies against synthetic human β-endorphin. β-endorphin in standards and samples compete with $^{125}$I-labelled β-endorphin in binding to the antibodies. $^{125}$I-β-endorphin binds to the antibodies in a reverse proportion to the concentration of β-endorphin in standards and samples. In order to increase the sensitivity of the assay a sequential incubation is performed. Antibody-bound $^{125}$I-β-endorphin is separated from the unbound fraction using the double antibody polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured. The antiserum used is specific for the N-terminal region of the β-endorphin with very low cross reaction with β-lipotropin.

2. Precautions

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- This radioactive material may be received, acquired, possessed and used only by physicians, laboratories or hospitals. Its receipt, acquisition, possession, use and/or transfer are subject to the regulations and a specific license issued by the Nuclear Regulatory Commission or issued by a State with which the Nuclear Regulatory Commission has entered into an agreement for the exercise of regulatory authority.
- All reagents of this testkit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- The assay reagents contain sodium azide or thimerosal which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
• Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidical soap and copious amounts of water.

• Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

• Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.

• Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.

• Do not use reagents beyond expiry date as shown on the kit labels.

• All in the protocol indicated volumes (pipetting volumes and pretreatment steps) has to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.

• Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

• Radioactive materials should be confined to specifically designated, regularly monitored areas in the laboratory, away from traffic and restricted to authorised personnel. Use disposable labware and disposable absorbent bench covers. Always wear film budges, lab coats and disposable gloves. Wipe up all spills immediately, cleaning the contaminated area with a decontaminant and dispose of the contaminated materials as radioactive waste.

• Safety Data Sheets for this product are available on the homepage of IBL or upon request directly from IBL. The Safety Data Sheets fit the demands of:
  - EU-Guideline 91/155 EWG
  - ISO-Standard 11014
  - ANSI-Standard
  - OSHA (US)
3. Kit Components

3.1 Contents of the Kit

The reagents provided in each kit are sufficient for 100 tubes.

3.1.1 Anti-β-Endorphin (Reagent A)
1 vial
Rabbit antiserum to synthetic, human β-endorphin (β-lipoprotein 61-91).
Lyophilised in 2.0 ml 0.5 M phosphate buffer, pH 7.4, 10 % human serum albumin and 0.5 % sodium azide. Colour: yellow.
For 100 tubes. Reconstitution in 22 ml distilled water.

3.1.2 \(^{125}\)I-β-Endorphin (Reagent B)
1 vial
1.5 µCi or 56 Kqb. Produced by iodination of synthetic, human β-endorphin. HPLC-purified, monoidinated. Specific activity: 1700-2100 µCi/nmol (62-77 Mbq/nmol).
Lyophilised in 2.5 ml 0.5 M phosphate buffer, pH 7.4, 10 % human serum albumin, 0.5 % sodium azide and 500 KIU Trasylol®/ml.
Contains 0.12 ml normal rabbit serum. Colour: blue
Reconstitution in 25 ml distilled water.

3.1.3 Double antibody-PEG (Reagent C)
1 vial
50 ml goat anti-rabbit-Ig antiserum. Contains 5 % (w/v) polyethylene glycol 6000.
3.1.4 Diluent (Reagent D) 1 vial
25 ml, buffer for dilution of the β-endorphin standard and reconstitution of sample extracts, 0.05 M phosphate, pH 7.4, 1.0 % human serum albumin, 0.05 % sodium azide and 500 KIU Trasylool®/ml.

3.1.5 β-endorphin standard (Reagent E) 1 vial
5.00 ml, 500 pmol/l (1731 pg/ml), synthetic human β-endorphin. Lyophilised in 0.05 M phosphate, pH 7.4, 1.0 % human serum albumin, 0.05 % sodium azide and 500 KIU Trasylool®/ml.
Reconstitution in 5.00 ml distilled water.

Conversion of pmol/l to pg/ml: pmol/l x 3.463 = pg/ml.

3.1.6 β-endorphin controls (Reagent F-G)
Lyophilized controls with two different levels. 1.0 ml of each control after reconstitution. See vial label for concentrations. They should be assayed directly without extraction.

Material required but not provided

- Distilled water
- Acetone pro analysi
- Acetic acid
- Hydrochloric acid, 0.2 M
- Sep-pak C 18 cartridges
- 15 ml conical glass tubes
- 11-13 x 55 mm disposable test tubes (polystyrene)
- NSB- and TOT-tubes
- Pipettes with disposable tips: 100, 200, 500 and 1000 µl
- Glass pipettes: 1.00, 5.00 ml
- Vortex mixer
- Equipment for evaporation under vacuum
- Centrifuge, refrigerated, giving minimum 1700 x g
- Gamma counter
3.2 Storage and Stability of the Kit

Store all reagents at 2-8 °C before reconstitution and use. Reagents B, E and F-G should be stored at -18 °C, or lower if reused. The stability of the reagents is found on the label of the vials. Reconstituted reagents are stable for 10 weeks or until the expiry date is reached.

3.3 Preparations of Reagents

Reagents should be reconstituted 30 minutes before use.

3.3.1 Reagent A: Anti-β-endorphin
Reconstitute with 22 ml distilled water. Store at 2-8 °C.

3.3.2 Reagent B: $^{125}$I-β-endorphin
Reconstitute with 25 ml distilled water. Store at -18 °C or lower if reused.

3.3.3 Reagent C: double antibody-PEG
Mix thoroughly before use. Store at 2-8 °C.

3.3.4 Reagent E: β-endorphin standard, 500 pmol/l
Reconstitute with 5.00 ml distilled water. Store at -18 °C or lower if reused.

3.3.5 Reagent F-G: Controls
Reconstitute with 1.00 ml distilled water. Store at -18 °C or lower if reused.
3.4 Storage and Stability of prepared Reagents

3.4.1 Diluent (Reagent D)
Store the Diluent at 2 - 8 °C for up to expiry date of the kit.

3.4.2 Control Sera
For longer storage freeze in aliquots at –20 °C until exp. date of the component.

3.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets (see chapter 3).

3.6 Damaged Test Kits

In case of any severe damage of the test kit or components, IBL have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be use for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.
4. Specimen

4.1 Collection

Plasma

The usual precautions for venipuncture should be observed. Do not use grossly hemolytic, icteric or grossly lipemic specimens.

Blood is collected in tubes containing EDTA and Trasylol® (5000 KIU Trasylol in a 10 ml vacutainer tube). The sample is cooled in an ice-bath immediately.

4.2 Storage

Plasma

Plasma is separated by centrifugation at + 4 °C. The plasma should be frozen within 1 hour and stored at - 20 °C or lower until assayed. Repeated freezing and thawing should be avoided.

4.3 Pretreatment

Extraction
The described extraction procedure is based on the use of Sep-pak® C 18 cartridges (available from Waters, M.A., USA cat. no. WAT 020515). The procedure involves concentration of β-endorphin with a factor of 10.

4.3.1 Thaw the plasma immediately before starting the extraction. Store at 2-8 °C before application on the sep-pak cartridge.

4.3.2 The sep-pak cartridge is activated by passing through it 2 ml of acetone.

4.3.3 Wash the cartridge twice with 2 ml 4 % acetic acid in distilled water.
4.3.4 Apply 3.00 ml plasma sample on the sep-pak cartridge. The flowrate should not exceed 3 ml per minute.

4.3.5 Wash the cartridge twice with 2 ml 4 % acetic acid in distilled water.

4.3.6 Elute the β-endorphin with 1.5 ml 0.2 M hydrochloric acid/acetone (25:75).

4.3.7 Dry the eluate under vacuum.

4.3.8 Dissolve the extracted β-endorphin in 300 µl diluent (Reagent D). Vortex mix and allow the sample to stay for 30 minutes before assay with the radioimmunoassay procedure.

**Recovery**

It is important that the recovery is controlled under the user’s own experimental conditions. For the determination of the recovery in the extraction procedure prepare a control as follows:

To 3.00 ml blood donor plasma add exactly 75 µl of β-endorphin standard 500 pmol/l. The concentration will be 12.5 pmol/l. Extract the control according to method description. Extract also the same amount of plasma without added β-endorphin for correction for endogenous β-endorphin in the plasma.
5. Test Procedure

GENERAL REMARKS:

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. The water used for reconstitution of lyophilised reagents should be distilled in an all-glass apparatus or be of corresponding purity.

Once the test has been started, all steps should be completed without interruption.

Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

5.1 Assay Procedure

5.1.1 Reconstitute the reagents according to the instructions.

5.1.2 Prepare the β-endorphin working standards by dilution of the 500 pmol/l standard (Reagent E) with the diluent (Reagent D) according to the following:

a) Reagent E after reconstitution = 500 pmol/l
b) 1.00 ml standard 500 pmol/l + 1.00 ml diluent = 250 pmol/l
c) 1.00 ml standard 250 pmol/l + 1.00 ml diluent = 125 pmol/l
d) 1.00 ml standard 125 pmol/l + 1.00 ml diluent = 62.5 pmol/l
e) 1.00 ml standard 62.5 pmol/l + 1.00 ml diluent = 31.3 pmol/l
f) 1.00 ml standard 31.3 pmol/l + 1.00 ml diluent = 15.6 pmol/l
g) Diluent = 0 pmol/l

Store the standard solutions at -20 °C or lower if reused.

5.1.3 Pipette 100 µl of standards a-g (0-500 pmol/l) and reconstituted sample extracts in their respective tubes. Pipette 100 µl of the zero-standard in the NSB-tubes.
5.1.4 Add 200 µl anti-ß-endorphin (Reagent A) to all tubes except the NSB- and TOT-tubes.

5.1.5 Add 200 µl diluent (Reagent D) to the NSB-tubes.

5.1.6 Vortex-mix and incubate for 18-24 hours at 2-8 °C.

5.1.7 Add 200 µl ¹²⁵I-ß-endorphin (Reagent B) to all tubes. The TOT-tubes are sealed and kept aside.

5.1.8 Vortex-mix and incubate for 18-24 hours at 2-8 °C.

5.1.9 Add 500 µl double antibody-PEG (Reagent C) to all tubes except the TOT-tubes (mix this reagent before pipetting).

5.1.10 Vortex-mix and incubate for 30-60 minutes at 2-8 °C.

5.1.11 Centrifuge the tubes for 15 minutes at +4 °C (1700 x g).

5.1.12 Decant the supernatant immediately after centrifugation.

5.1.13 Count the radioactivity of the precipitates in a gamma counter (counting time 2 minutes).

Important notes:
Add each aliquot of reagent to the bottom of coated tubes to ensure complete mixture of reagents.
The washing step is an important part of the total assay procedure. Thorough and complete aspiration of the wash solution is essential for the precision of the assay.
5.2. Calculation of Results

Subtract the average count rate (CPM) of the non-specific binding from the count rate (CPM) of the replicates of standards, controls and samples.

A standard curve is generated by plotting the precipitated CPM, bound fraction (in CPM or %B/TOT) against the concentration of the β-endorphin-standards.

To obtain the β-endorphin concentrations in the samples and controls read the corresponding concentrations to their precipitated CPM or %B/TOT from the generated standard curve.

The received concentrations are corrected for the concentration factor in the extraction procedure. According to the method described the β-endorphin is concentrated 10 times. Divide with 10. Finally the found concentration is corrected for the recovery of the recovery controls.

The standard curve and the calculation of the concentrations in samples can also be done by a computer method. A spline method may be used.

For the accuracy of the results obtained see chapter 7 (Assay Characteristics).
Below is listed a typical example of a standard curve with the β-Endorphin RIA.

5.3 Automation

Up to now, no protocol for the automatic run of the β-Endorphin RIA is available.
6. Assay characteristics

6.1 Expected values

Plasma from 27 healthy blood donors was assayed with these reagents. In 26 samples the concentrations were <3 pmol/l (10 pg/ml). In 1 sample the concentration was 5 pmol/l (17 pg/ml).

6.2 Specificity

The following cross reactions have been found:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity (%)</th>
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<tbody>
<tr>
<td>ß-endorphin</td>
<td>100</td>
</tr>
<tr>
<td>ß-lipotropin</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>α-endorphin</td>
<td>69</td>
</tr>
<tr>
<td>Met-enkephalin</td>
<td>9</td>
</tr>
<tr>
<td>Leu-enkephalin</td>
<td>&lt; 0,04</td>
</tr>
<tr>
<td>ß-lipotropin 61-87</td>
<td>43</td>
</tr>
<tr>
<td>ß-lipotropin 61-69</td>
<td>67</td>
</tr>
<tr>
<td>ß-lipotropin 66-91</td>
<td>2</td>
</tr>
<tr>
<td>ß-lipotropin 88-91</td>
<td>&lt; 0,04</td>
</tr>
</tbody>
</table>

6.3 Sensitivity

The sensitivity calculated from a decrease in binding of 2 SD in the zerostandard is 3 pmol/l or 10 pg/ml (recovery = 70 %). The sensitivity calculation is based upon a concentration with a factor of ten in the extraction procedure.
6.4. Precision

6.4.1 Intra Assay Variation

The Intra assay variation amounts to 7.1 %.

6.4.2 Inter Assay Variation

The Inter assay variation amounts to 7.2 %.

6.5 Accuracy

6.5.1 Recovery

The mean recovery in the extraction procedure is 80-90 %.

6.5.2 Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The kit controls and the corresponding results of the IBL QC-Laboratory are stated in the QC certificate added to the kit. The values stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or IBL directly.
Calculate for each assay the % bound activity in the zerostandard (Bo/TOT x 100) and the non-specific binding (NSB/TOT x 100). Bo/TOT x 100 is generally between 35-50 % at the reference date and may have decreased a few % at the expiry date of the kit. The non-specific binding is less than 6 %. As a good control routine it is recommended to analyse two known plasma samples (controls) in each assay. Plasma pools with known concentrations of β-endorphin should be stored at -25 °C or lower.
7. Limitations of Use

7.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters ‘Specimen - Collection’.

Azide and thimerosal at concentrations higher than 0.1 % interfere in this assay. Therefore control sera or samples containing higher concentrations of the above mentioned components may give false results.

7.2 High-Dose-Hook Effect

There exists no High-Dose-Hook effect in a competitive assay. In case of sandwich assays the probability of a High-Dose-Hook effect is reduced if the antibody and antigen containing solutions are added in a sequential order.

8. Legal Aspects

8.1 Reliability of Results

The test must be performed exactly as per the manufacturer’s instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL.
8.2 Complaints

Complaints will only be accepted in written format (preferably on the manufacturer's complaint form) and only if all details of the test kit, as well as the test results, are included. A copy of the complaint form is available from IBL upon request.

8.3 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

8.4 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject to point 9.3. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.
9. References

1. Bramnert, Ml, Ekman, R., Larsson, t. and Thorell, J.I.
Characterization and application of a radioimmunoassay for β-endorphin using an antiserum with negligible cross reactivity against β-lipotropin.

2. Winther Bach, F., Ekman, R. and Jensen, M.
β-endorphin-immunoreactive components in human cerebrospinal fluid.
Regulatory Peptides, 16:189-198 (1986)

Plasma β-endorphin during clinical and experimental pain.

4. Hardebo, J.E. and Ekman, R.
Substance P and opioids in cluster headache.

5. Angwin, P. and Barchas, J.D.
Use of silicic acid extraction and reversed-phase columns for rapid purification prior to radioimmunoassay
LIABILITY: Complaints will be accepted in each mode – written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols  /  Symbole  /  Symbôles  /  Símbolos  /  Σύμβολα