Myelin Basic Protein (MBP) RIA

For the quantitative determination of MBP in cerebrospinal fluid (CSF)

*Please read carefully due to Critical Changes, e.g., assay procedure*

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 24-MBPHU-R50, R100
Size: 50 determinations, 100 determinations
Version: ALPCO April 1, 2011
I. INTENDED USE

The Myelin Basic Protein (MBP) RIA (I-125 Radioimmunoassay) kit measures MBP in cerebrospinal fluid (CSF). It is for research use only. It is not for use in diagnostic procedures.

II. INTRODUCTION

MBP constitutes about one-third of the proteins in myelin sheath and appears to be specific for myelin (1). It consists of a single chain of 170 amino acid residues (molecular weight: 18,400 Da) whose sequence is very similar in different species (1, 2).

Elevated concentrations of MBP in CSF have been found in individuals with active multiple sclerosis (3, 4), following neurosurgery (5) and head trauma (6, 7), and with acute stroke (8). It has been claimed that MBP determination in CSF is valuable in following the activity of multiple sclerosis in its acute phases (9, 10) and also as a marker for the severity of brain damage in head trauma (7) and acute stroke (8). It has also been shown that determination of MBP in CSF may be of significance in the study of necrotizing leukoencephalopathy in children or young adults with acute lymphoblastic leukemia (11). It may serve as an index of the severity of disease in individuals with neuro-Bechet's disease (12) and in individuals with encephalitis of various origins (13).

III. PRINCIPLE

This double antibody MBP procedure is a competitive RIA in which I-125 labeled MBP competes with MBP in samples for limited antibody binding sites. After incubation for a fixed time, separation of bound from free is achieved by the polyethylene glycol accelerated double antibody precipitation method. The radioactivity of the precipitated antibody-bound complex is counted in a gamma spectrometer. The concentration of MBP in the samples is determined from a calibration curve.

IV. WARNING AND PRECAUTIONS

• Some of the reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation. Sodium azide is also toxic. Care should be taken to avoid ingestion.

• Handle all components and all samples as if capable of transmitting hepatitis and acquired immunodeficiency syndrome. Source materials derived from human body fluids used in the preparation of this kit were tested and found negative for hepatitis B surface antigen and HIV antibody. However, no known test can guarantee that such material does not contain the causative agent of viral hepatitis or HIV antigen.

• Caution - radioactive material not for use in humans or animals

To minimize exposure to radiation, the user should adhere to guidelines set forth in the National Bureau of Standards publication on the Safe Handling of Radioactive Materials (Handbook No. 92, issued March 9, 1964) and in subsequent publications issued by state and federal authorities. Radioactive materials should be confined to specifically designated, regularly monitored areas in the laboratory, away from traffic, and restricted to authorized personnel. Food, drink, smoking, and the application of cosmetics should all be expressly prohibited. Use disposable labware and disposable absorbent bench covers. Always wear film badges, lab coats, and disposable gloves. Never pipet radioactive materials by mouth. Wipe up spills promptly, washing the affected surface with a decontaminant and monitoring with a radiation detector. Place contaminated tissues, tubes, bench covers, gloves, etc., in a specially marked container for disposal as solid...
radioactive waste. Wash thoroughly after work. Maintain complete records of the receipt, use, and disposal of all radioactive materials. Discard liquid, dispersible, and solid radioactive waste only as permitted by federal, state, and local ordinances.

V. REAGENTS AND MATERIALS
Materials supplied in this kit are sufficient for 50 (24-MBPHU-R50) or 100 (24-MBPHU-R100) determinations.

- MBP Calibrators: Part No.: 16-11, 16-12, 16-13, 16-14, 16-15, and 16-16. Buffered reagents containing five different concentrations of MBP. Concentrations (ng/ml) are indicated on the vials (approximately 1.0 - 20.0 ng/ml). Sodium azide (0.01%) is added as a preservative. Reconstitute calibrator 1 (0 ng/ml) with 1 ml of distilled water and the other calibrators with 0.5 ml of distilled water per vial. (24-MBPHU-R50 - 1 of each vial, 24-MBPHU-R100 - 2 of each vial)

- MBP Controls: Part No.: 16-21 and 16-22. Cerebrospinal fluid containing low and high levels of MBP. Concentration ranges (ng/ml) are indicated on the vials. Sodium azide (0.01%) is added as a preservative. Reconstitute with 0.5 ml of distilled water per vial. (24-MBPHU-R50 - 1 of each vial, 24-MBPHU-R100 - 2 of each vial)

- I-125 MBP Reagent: Part No.: 16-30. Buffered reagent containing I-125 MBP tracer. 0.01% sodium azide added as a preservative. < 300 kBq (8 µCi). Reconstitute with 3.0 ml of distilled water. Green color. (24-MBPHU-R50 - 1 vial, 24-MBPHU-R100 - 2 vials)

- MBP Antiserum Reagent: Part No.: 16-40. Buffered reagent containing anti-human MBP antiserum. 0.01% sodium azide added as a preservative. Yellow color. (24-MBPHU-R50 - 5 ml, 24-MBPHU-R100 - 10 ml)

- MBP Buffer Reagent: Part No.: 16-60. Protein based buffer reagent containing 0.01% sodium azide as a preservative. (24-MBPHU-R50 - 5 ml, 24-MBPHU-R100 - 10 ml)

- Precipitating Reagent: Part No.: 11-50. Buffered reagent containing gamma globulin and dilute polyethylene glycol. 0.01% sodium azide is added as preservative. (24-MBPHU-R50 - 50 ml, 24-MBPHU-R100 - 100 ml)

VI. STORAGE AND STABILITY
a. The unopened test kit is stable until the expiration date shown on the kit label.
b. Store the unopened test kit in refrigerator (2-8°C).
c. Reconstituted I-125 MBP reagent, MBP calibrators, and MBP controls should be stored frozen (-20°C or lower).
d. MBP antiserum reagent, precipitating reagent, and MBP buffer reagent should be stored at 2-8°C.

VII. SPECIMEN COLLECTION
Collect 1-2 ml CSF in a plastic tube and remove any insoluble material by centrifuging cold at
2,000 RPM for five minutes. The procedure calls for 100 μl of CSF per assay tube. The samples can be stored under refrigeration (2-8°C) for 24 hours; if they are to be kept for a longer period of time the samples should be frozen (-20°C or lower). Divide the samples into aliquots before freezing to avoid repeated freeze-thaw cycles. Any CSF contaminated with blood should be spun down or not used.

VIII. TEST PROCEDURE

Table 1. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Material</th>
<th>Part No.</th>
<th>24-MBPHU-R50 Quantity</th>
<th>24-MBPHU-R100 Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP calibrator 1</td>
<td>16-11</td>
<td>One vial, 1 ml (lyophilized)</td>
<td>Two vials, 1 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP calibrator 2</td>
<td>16-12</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP calibrator 3</td>
<td>16-13</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP calibrator 4</td>
<td>16-14</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP calibrator 5</td>
<td>16-15</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP calibrator 6</td>
<td>16-16</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP control level I</td>
<td>16-21</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP control level II</td>
<td>16-22</td>
<td>One vial, 0.5 ml (lyophilized)</td>
<td>Two vials, 0.5 ml (lyophilized)</td>
</tr>
<tr>
<td>I-125 MBP reagent</td>
<td>16-30</td>
<td>One bottle, 3 ml (lyophilized)</td>
<td>Two bottles, 3 ml (lyophilized)</td>
</tr>
<tr>
<td>MBP antiserum reagent</td>
<td>16-40</td>
<td>One bottle, 5 ml</td>
<td>One bottle, 10 ml</td>
</tr>
<tr>
<td>MBP buffer reagent</td>
<td>16-60</td>
<td>One bottle, 5 ml</td>
<td>One bottle, 10 ml</td>
</tr>
<tr>
<td>Precipitating reagent</td>
<td>11-50</td>
<td>One bottle, 50 ml</td>
<td>One bottle, 100 ml</td>
</tr>
</tbody>
</table>

**Materials required but not supplied:**
- Gamma counter
- Centrifuge capable of >1,500 x g (preferably refrigerated)
- Refrigerators (2-8°C and -20°C)
- Vortex mixer
- Test tube racks
- Deionized or distilled water
- Repeating dispenser for 1.0 ml (optional)
- Foam decanting rack
- 12 x 75 mm disposable plastic test tubes
- 100 μl precision pipet with disposable tips
- Volumetric pipets 1.0 ml and 10.0 ml
**Assay Procedure**

All components except the precipitating solution must be at ambient temperature before use. Keep precipitating reagent cold.

1. Label and arrange **plastic test tubes** in duplicate according to the protocol shown in Table 2.
2. Pipet 100 μl of the calibrators, controls, and samples to the appropriate tubes, except the total count tubes. Add 200 μl of calibrator 1 to the NSB (non-specific binding) tubes.
3. Add 100 μl of MBP buffer reagent to all the tubes, except the total count tubes.
4. Add 100 μl of MBP antiserum reagent to all tubes, except the NSB and total count tubes. Vortex.
5. Incubate at room temperature for 2 hours.
6. Add 50 μl of I-125 MBP reagent to all tubes. Vortex.
7. Incubate at room temperature for 2 hours.
8. Add 1.0 ml of cold precipitating reagent to all tubes, except the total count tubes. Vortex and let stand for 20 minutes.
9. Centrifuge all tubes except the total count tubes for 25 minutes at 1,500 x g, preferably at 2-8°C.
10. Using a foam decanting rack decant all tubes, except the total count tubes. Let the tubes stand inverted on absorbent paper for 30-60 seconds. Tap the tubes gently and blot the rims to remove all residual droplets.
11. Count each tube in a gamma counter for 1 minute.

Table 2. ASSAY PROTOCOL

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Calibrator/Control/Sample</th>
<th>MBP buffer reagent</th>
<th>MBP antiserum reagent</th>
<th>I-125 MBP reagent</th>
<th>Precipitating reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μl</td>
<td>μl</td>
<td>μl</td>
<td>μl</td>
<td>ml</td>
</tr>
<tr>
<td>1, 2</td>
<td>Total Count</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>3, 4</td>
<td>NSB [calibrator 1]</td>
<td>200</td>
<td>100</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>5, 6</td>
<td>Maximum binding (Bo) [calibrator 1]</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>7, 8</td>
<td>calibrator 2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>9, 10</td>
<td>calibrator 3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>11, 12</td>
<td>calibrator 4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>13, 14</td>
<td>calibrator 5</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>15, 16</td>
<td>calibrator 6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>
**Short Protocol**

- Pipet 100 µl calibrators, controls, and samples. Pipet 200 µl calibrator 1 to NSB tubes.
- Pipet 100 µl MBP buffer reagent to all tubes, except total count tubes.
- Add 100 µl MBP antiserum reagent to all tubes, except total count and NSB tubes.
- Vortex.
- Incubate for 2 hours at RT.
- Add 50 µl I-125 MBP reagent to all tubes.
- Vortex.
- Incubate for 2 hours at RT.
- Add 1 ml of cold precipitating reagent, except the total count tubes.
- Vortex and let stand for 20 minutes.
- Centrifuge all tubes except total count tubes for 25 minutes at 1,500 x g, preferably at 2-8°C.
- Decant all tubes for 30-60 seconds, except total count tubes.
- Count for 1 minute.

**Calculation of results:**

1. Determine average counts per minute (cpm) for NSB, calibrators, controls, and sample tubes.
2. Calculate percent tracer bound (%B) for each calibrator, control, and sample relative to the maximum binding (B₀, calibrator 1, 0 ng/ml) tubes as follows:

\[
\%B/B₀ = \frac{cpm \text{ (calibrator, control, or sample)} - cpm \text{ (NSB)}}{cpm \text{ (calibrator 1)} - cpm \text{ (NSB)}} \times 100
\]

Using Logit-Log graph paper, plot percent bound on the vertical axis against concentration on the horizontal axis for each of the calibrators and draw a best fit straight line through the points. Alternatively, the data can be plotted on linear-log graph paper by plotting percent bound on the vertical axis against concentration on the horizontal axis for each of the calibrators and drawing a best fit straight line through the points. MBP concentration for the samples may then be estimated from the line by interpolation.

**Quality control**

The reliability of test results should be monitored by the routine use of control reagents of known MBP concentrations. Two quality control pools are supplied with the kit and should be analyzed with each assay. The results should be charted from assay to assay and the overall performance checked periodically.

**IX. LIMITATIONS**

1. Samples with values greater than the highest calibrator should be diluted 10 times with calibrator 1 (0 ng/ml) and assayed again.
2. Serial dilution of CSF samples does not parallel the standard curve.
3. This kit is designed to measure MBP in CSF samples. Any CSF contaminated with blood should either be spun down or not assayed.

X. PERFORMANCE AND CHARACTERISTICS

- **Precision:**
The reliability of the MBP RIA test kit procedure was assessed by examining its reproducibility on samples selected to represent a range of MBP levels. Coefficient of variation between and within the assay was less than ten percent.

- **Sensitivity:**
Ten calibrator 1 (maximum binding) tubes were processed along with a calibration curve. Mean and standard deviation were calculated for the counts per minute of the ten calibrator 1 tubes. Apparent sensitivity was then calculated from the average of 1, 2, 3 and 4 standard deviations and was found to be 1.0 ng/ml.

- **Recovery:**
Recovery of known quantity of MBP added to predetermined CSF samples ranged from 85 – 115 percent.

- **Parallelism:** Serial dilution of CSF MBP samples ranged from 80-120 percent.

XI. EXPECTED VALUES
Normal range study was conducted on fifty human CSF samples using this MBP double antibody RIA test kit. 95% of the samples had MBP values less than or equal to 3.0 ng/ml. The highest value observed was 3.8 ng/ml. The normal range limit suggested by this study should be regarded only as a guideline. It is important that each laboratory should establish its own normal values that are representative of the characteristic of the population that is being tested.

Table 3. TYPICAL STANDARD CURVE DATA

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Net Average Counts</th>
<th>Net Average B/B₀ (%)</th>
<th>Conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>94833</td>
<td>-</td>
<td>TC</td>
</tr>
<tr>
<td>3,4</td>
<td>1571</td>
<td>-</td>
<td>NSB</td>
</tr>
<tr>
<td>5,6</td>
<td>39496</td>
<td>-</td>
<td>B₀</td>
</tr>
<tr>
<td>7,8</td>
<td>36684</td>
<td>92.58</td>
<td>1.0</td>
</tr>
<tr>
<td>9,10</td>
<td>32484</td>
<td>81.51</td>
<td>2.0</td>
</tr>
<tr>
<td>11,12</td>
<td>24059</td>
<td>59.30</td>
<td>5.0</td>
</tr>
<tr>
<td>13,14</td>
<td>17402</td>
<td>41.74</td>
<td>10.0</td>
</tr>
<tr>
<td>15,16</td>
<td>9256</td>
<td>20.26</td>
<td>20.0</td>
</tr>
</tbody>
</table>
Logit (B/B₀)  
Net% B₀ = 39.99  
%NSB = 1.66  
ED (20%) = 21.00  
ED (50%) = 7.00  
ED (80%) = 2.33

XII. REFERENCES