AnshLabs

MBP ELISA

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AL-108

INTENDED USE

The myelin basic protein (MBP) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of MBP in cerebrospinal fluid. This kit is intended for research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Myelin is the insulating sheath which surrounds neurons. In the central nervous system 30% of the myelin is composed of Myelin Basic Protein.[1] the function of MBP is not completely defined, although it may provide structural support. Human MBP is an 18.5 kDa amino acid monomeric protein. The structure of MBP can be divided into 3 segments joined by phenylalanine doublets: A—residues 1-43; B—residues 44-89; and C—residues 90-170.[2] Segments A and C, the N- and C-termini of the protein, respectively, are highly homologous. Myelin immunoreactivity in cerebrospinal fluid (CSF) is generally due to the B-segment; A and C segments are usually present in low or undetectable levels.[3, 4]

PRINCIPLE OF THE TEST

The MBP ELISA is an enzymatically amplified three step sandwich- type immunoassay. In the assay, Calibrators, Controls and Unknown CSF samples are incubated in micro wells, which have been coated with MBP antibody. After incubation and washing, the wells are incubated with biotinylated detection antibody. After a second incubation and washing step, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of MBP in the samples and calibrators.

MATERIALS SUPPLIED

CAL-108A - CAL-108F MBP Calibrator A-F (Lyophilized)

Six vials, labeled A-F, containing concentrations of approximately 0, 0.3-11 ng/mL human MBP in protein-based buffer containing serum and Sodium Azide. Refer to **calibration card** for exact concentrations. Store unopened at 2 – 8°C until the expiration date. Reconstitute calibrators A-F with 1 mL deionized water. Mix well and use immediately after reconstitution. **Discard after use**.

The analyte concentration in the MBP calibrators is traceable to the manufacturer's working calibrators. The assigned values are specific to the assay methodologies. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias. Calibrators are shipped ambient.

CTR-108-I & CTR-108-II MBP Controls I &II (Lyophilized)

Two vials, 1 mL each, labeled Levels I and II containing low and high MBP concentrations in protein-based buffer containing serum and Sodium Azide. Refer to **calibration card** for exact concentrations. Store unopened at 2 – 8°C until the expiration date. Reconstitute each vial with 1 mL deionized water. Mix well, and use immediately after reconstitution. **Discard after use.**

PLT-108 Anti-MBP Antibody Coated Microtitration Strips

One strip holder, containing 96 polystyrene microtitration wells with mouse monoclonal anti-human MBP immobilized to the inside wall of each well. Store at 2 - 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

BCC-108 MBP Biotin Conjugate Concentrate:

One vial, 0.4 mL containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Dilute prior to use in MBP Conjugate diluent. Store at 2 - 8°C until expiration date

CND-208 MBP Conjugate Diluent

One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2 – 8°C until expiration date.

SAP-108 MBP Streptavidin-Enzyme Conjugate—Ready-to-Use (RTU):

One amber bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based (BSA) buffer and a non-mercury preservative. Store at 2°C -8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 – 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

WARNING: Potential Biohazardous Material

This reagent may contain some animal source material (e.g. serum) or materials used in conjunction with animal source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious animal material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 6th Edition, 2020[5].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin™ 300 and Sodium Azide[6] as a preservative. ProClin™ 300 and Sodium Azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Cerebrospinal fluid is the recommended sample type.

b) Avoid assaying lipemic, hemolyzed or icteric samples.

c) Each laboratory should determine the acceptability of its own CSF collection, shipping, and storage methods.

d) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the MBP ELISA. It is the responsibility of the customer to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix different lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the enzyme conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

Preparation of Reagents

1. **MBP Calibrator A-F and MBP Controls I & II:** Tap and reconstitute MBP Calibrator A-F and MBP Controls I & II each with 1 mL deionized water. Mix well and use immediately after reconstitution.
2. **Wash Solution:** Prepare Wash Solution by diluting Wash Concentrate A **25-fold** with deionized water. The Wash Solution is stable for one (1) month at room temperature when stored in a tightly sealed bottle.
3. **MBP Antibody-Biotin Conjugate Solution:** The MBP Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of MBP Conjugate Diluent, according to the number of wells used. If an entire plate is to be used pipet exactly 220 µL of the Concentrate into 11 mL of the buffer.
4. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the re-sealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

Allow all samples and reagents to reach room temperature. Mix reagents thoroughly by gentle inversion before use. Calibrators, controls and samples should be assayed in duplicate.

***NOTE**: If the concentration of MBP in the sample is greater than the highest calibrator, dilute appropriately using MBP in spinal fluid with negligible MBP concentration or Calibrator A.*

1. Mark the microtitration strips to be used.
2. Pipet **100 µL** of the calibrators, controls and samples to the appropriate wells.
3. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **1 hour** at room temperature (23 ± 2°C).
4. Prepare the Antibody-Biotin Conjugate Solution as described under the "Preparation of Reagents" section of this package insert.
5. Aspirate and wash each well **5 times** with the wash solution **(350 µL/per well)** using an automatic microplate washer.
6. Add **100 µL** of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
7. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **1 hour** at room temperature (23 ± 2°C).
8. Aspirate and wash each well **5 times** with the wash solution **(350 µL/per well)** using an automatic microplate washer.
9. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
10. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
11. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.
12. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
13. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    NOTE: Visually monitor the color development to optimize the incubation time.
14. Add **100 µL** of the stopping solution to each well using a repeater pipette. Read the absorbance of the solution in the wells **within 20 minutes,** using a microplate reader set to **450 nm.**
    ***NOTE**: Zero calibrator should be programmed as "**Blank**" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm**.*

RESULTS

NOTE: *The results in this package insert were calculated by plotting the* ***log optical density (OD) data on the y-axis and log MBP concentration on X-axis*** *using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Optimum results can be obtained at incubation temperature of **(23 ± 2°C)**.
2. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the MBP concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the MBP concentrations of the Controls and Unknowns from the calibration curve by matching their mean OD readings with the corresponding MBP concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (Calibrator A) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor, if required.

LIMITATIONS

The reagents supplied in this kit are optimized to measure MBP levels in human CSF and other biological fluids. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophilic antibodies in the samples[7].

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable control ranges to ensure proper performance.
- MBP ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for MBP ELISA controls are printed on the **calibration card**.
- A full calibration curve, low and high level controls, should be included in each assay.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean Absorbance	Conc. (ng/mL)
	Calibrators		
A1, A2	A	0.063 **(Blank)**	0
B1, B2	B	0.037	0.35
C1, C2	C	0.123	0.8
D1, D2	D	0.392	1.8
E1, E2	E	1.281	4.2
F1, F2	F	3.671	10.5

CAUTION*: The above data must not be employed in lieu of data obtained by the user in the laboratory*

ANALYTICAL CHARACTERISTICS

Limit of Detection (LoD):

The lowest amount of MBP in a sample that can be detected with a 95% probability (n=24) is 0.093 ng/mL. The value was determined by processing four CSF samples in the range of 0.66 to 2.58 ng/mL following CLSI EP17 guidelines. Six assay runs were performed with all samples run in quadruplets per run.

Imprecision

Reproducibility of the MBP assay was determined in a study using three CSF pools. The study included a total of 6 assays, four replicates of each per assay (n=24). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the following table.

Sample	Mean Conc.	Within Run		Between Run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Pool-1	0.661	0.039	5.93%	0.022	3.39%	0.045	6.84%
Pool-2	1.745	0.027	1.56%	0.045	2.60%	0.053	3.03%
Pool-3	2.046	0.040	1.94%	0.034	1.67%	0.052	2.56%

Recovery:

Known amounts of MBP were added to two samples containing different levels of endogenous MBP. The concentration of MBP was determined before and after the addition of exogenous MBP and the percent recovery was calculated.

Sample	Endogenous Conc. (ng/mL)	Expected Conc.(ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	0.752	1.638	1.787	109
2	1.358	2.189	2.228	102

Linearity

Based on NCCLS EP-6-P multiple dilutions of the three serum samples containing various MBP levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	1:2	8.932	N/A	N/A
	1:4	4.466	4.121	92%
	1:8	2.233	2.261	101%
	1:16	1.117	1.173	105%
	1:32	0.558	0.57	102%
2	1:80	9.503	N/A	N/A
	1:160	4.252	4.315	101%
	1:320	2.126	2.254	106%
	1:640	1.063	1.200	113%
3	1:25	5.092	N/A	N/A
	1:50	2.546	2.596	102%
	1:100	1.273	1.387	109%
	1:200	0.637	0.750	118%
	1:400	0.318	0.353	111%

Analytical Specificity:

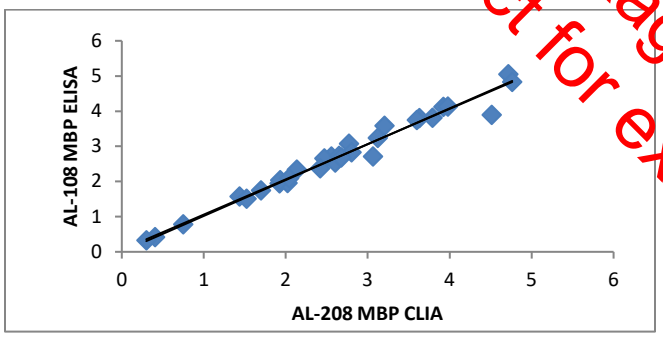
This monoclonal antibody pair used in the assay detects human MBP and does not cross-react with Bovine MBP at 20 ng/mL concentration. Specificity to other species has not been determined.

Interference:

When potential interferents (hemoglobin, biotin, triglycerides, and bilirubin) were added at specified concentration to control sample, MBP concentration were within ± 10% of the control as represented in the following table.

Interferent	Interferent Dose	Sample MBP (ng/mL)	Dosed Sample MBP (ng/mL)	MBP Difference (ng/mL)	% Difference to Reference
Hemoglobin	5 mg/mL	2.51	2.40	-0.11	-4.4
	2.5 mg/mL	2.61	2.54	-0.07	-2.8
	1 mg/mL	2.62	2.63	0.01	0.4
	0.1 mg/mL	2.73	2.63	-0.09	-3.4
Biotin	1200 ng/mL	2.28	2.25	-0.03	-1.5
	600 ng/mL	2.47	2.56	0.09	3.8
	200 ng/mL	2.67	2.66	-0.01	-0.4
Intralipids	20 mg/mL	2.54	2.58	0.05	1.8
	10 mg/mL	2.63	2.70	0.07	2.7
Bilirubin	0.66 mg/mL	2.64	2.59	-0.05	-2.1
	0.2 mg/mL	2.61	2.65	0.04	1.6

Method Comparison:
The Ansh MBP ELISA has been compared to AnshLite™ MBP CLIA (Method A) using 30 CSF samples. Analysis of the results yielded the following Regression:
Ansh MBP ELISA (AL-108) = 1.00 (Method A) + 0.06
(r=0.99; P<0.0001)



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Not for use in-vitro diagnostic procedures.

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