ELISA Kit Components	Amount	Part No.
MBP Coated Microwell Strip Plate	8-well strips (12)	630-101
Anti-MBP Positive Control	2 vials	630-122
Anti-Human IgM HRP Conjugate (100X)	0.15 ml	____
Sample Diluent Concentrate (20X)	10 ml	SD-20TG
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-630-130-MBP

PRECAUTIONS AND SAFETY INSTRUCTIONS

Controls, Sample Diluent, and Anti-human Ig-HRP contain ProClin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

*Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.*

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

LIMITS OF THE ASSAY

Quantitation of Antibody in a Sample

The ELISA measures anti-MBP activity, a combination of antibody concentration and avidity for the MBP antigen. Antibodies with substantially different IgG concentrations may display similar anti-MBP activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of Ig (e.g., ug/ml).

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (dilutions of the Positive Control), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in MBP avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the Positive Control or a dilution thereof (see Calculation of Results).

Non-specific Binding and Blocking of Antibody Activity

Antigen blocking is a strategy to verify that signal in the assay is due to specific anti-MBP antibody activity, rather than non-specific binding of non-anti-MBP Ig to the microwell. It is observed that antigen blocking of higher avidity antibodies is often more complete than with lower avidity antibodies, due to both antigen composition and antibody affinities. Therefore, the percent blocking of samples may vary from that of the Positive Control, and will rarely be 100% complete in an ELISA. For positive samples that display low % blocking, it is recommended they be comparatively tested on non-antigen coated microwells.

References: Berger T (2003) N Engl J Med. 349:139-45; Reindl M (1999) Brain,122:2047-2056; Egg R (2001) Mult Scler. 7:285-289; Ponomarenko, NA et al (2006) PNAS, 103(2): 281 - 286. Hock KS et al (2008) Pigment Cell Melanoma Res., 21(6):665-76.

Instruction Manual No. M-630-135-MBP

Human Anti-Myelin Basic Protein (MBP) IgM

ELISA Kit # 630-135-MBP

For Semi-Quantitative Determination of Anti-MBP IgM in Serum or Plasma

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

INTENDED USE

The Human Anti-MBP IgM ELISA Kit is an immunoassay suitable for detecting or titering total anti-MBP IgM specific for myelin basic protein (MBP) in Human serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.

INTRODUCTION

Myelin is a dielectric (electrically insulating) material that forms a layer, the myelin sheath, usually around only the axon of a neuron. It is essential for the proper functioning of the nervous system. Myelinated axons are white in appearance, hence the "white matter" of the brain. Myelin is composed of about 80% lipid and about 20% protein. Some of the proteins that make up myelin are myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP). When myelin degrades, conduction of signals along the nerve can be impaired or lost and the nerve eventually withers. Demyelination is the loss of the myelin sheath insulating the nerves, and is the hallmark of some neurodegenerative autoimmune diseases, including multiple sclerosis (MS), acute disseminated encephalomyelitis, transverse myelitis, Alexander's disease, chronic inflammatory demyelinating polyneuropathy, and Guillain-Barré Syndrome.

Knockout mice deficient in MBP have been developed which showed decreased amounts of central nervous system (CNS) myelination and a progressive disorder characterized by tremors, seizures, and early death. The pool of MBP in the CNS is very diverse, with several splice variants being expressed and a large number of post-translational modifications on the protein, which include phosphorylation, methylation, deamidation and citrullination. In multiple sclerosis, the most common disabling neurologic disease in young adults, antibodies against MBP are present in early stages. Another potential target antigen for autoreactive antibodies might be myelin oligodendrocyte glycoprotein (MOG), which is specific to the CNS and is located exclusively on the surface of myelin sheaths and oligodendrocytes. In experimental animal models of MS, demyelinating antibody responses are directed against MOG and MBP. A significant number of MS cases is characterized by the presence in the blood of autoantibodies against myelin protein components. Autoantibodies to MBP and MOG were proposed as biomarkers for clinical prognosis of MS. Similar Ig's were also found in mice with induced experimental allergic encephalomyelitis (EAE), which is an animal model of MS.

PRINCIPLE OF THE TEST

The Human anti-MBP Ig's ELISA kit is based on the binding of anti-MBP in samples to MBP immobilized on the microwells, and anti-MBP antibody is detected by specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-MBP Ig's present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of Human antibody in samples is calculated relative to anti-MBP Positive Control antibody.

CALCULATION OF RESULTS (continued)

II. Antibody Activity Units (Titer)

When the dilution curves of samples are not parallel to the Calibrator curve, antibody potency can be expressed in semi-quantitative activity units, using one of the Calibrators (i.e., dilution of the Positive Control) closest to 1.0 OD as the Index:

1. Calculate the mean net ODs for replicate samples and the selected Calibrator.
2. Divide each sample OD value by the Calibrator OD value, and multiply by the sample dilution and the Calibrator (U/ml) value = **Total Activity Units**

Typical Results: see Data Table in Section I.

1.06 [Sample, net OD] ÷ **1.24** [50 U/ml Calibrator, net OD] x **100** dilution x **50** U/ml = **4.1 k** Activity Units in serum.

III. Positive Index

Values of **Experimental** samples may be expressed relative to the values of **Control** samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the mean + 2 SD of the Control samples = Positive Index.
2. Divide each sample value by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are negative for antibody.

Typical Results:

	ELISA OD Units		Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.243	2.358	0.49	**4.79**
2	0.351	0.597	0.71	**1.21**
3	0.286	1.421	0.58	**2.89**
4	0.357	1.268	0.73	**2.58**
5	0.512	0.857	**1.04**	**1.74**
6	0.342	1.296	0.70	**2.63**
7	0.298	0.608	0.61	**1.24**
8	0.285	0.369	0.58	0.75
9	0.157	0.864	0.32	**1.76**
10	0.187	0.543	0.38	**1.10**
Mean	0.302			
SD	0.095			
Mean +2 SD	**0.492**	= Positive Index		

CALCULATION OF RESULTS

Several data reduction methods may be considered to optimize precision and to best represent the relationships among experimental and control groups.

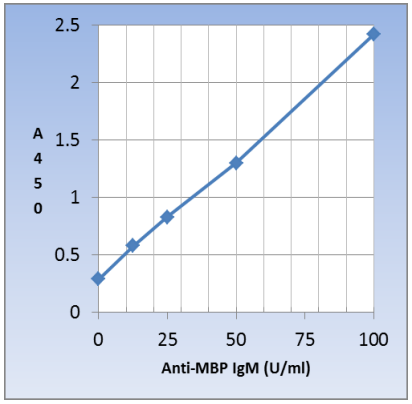
I. Use of a Calibrator Curve

When the dilution curves of samples are parallel to the Positive Control dilution curve (see Limits of the Assay), the anti-MBP activity units may be determined by interpolation from such a Calibrator curve, as follows:

1. The results may be calculated using any immunoassay software package. If software is not available, anti-MBP activity concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples. On graph paper plot the mean OD of the calibrators (y-axis) against the concentration (U/ml) of anti-MBP (x-axis). Draw the best fit curve through these points to construct the calibrator curve. A point-to-point construction is most common and reliable.
3. The anti-MBP activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample. Samples producing signals higher than the 100 U/ml calibrator should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators & Samples	A450 nm	Ig U/ml
A1, A2	**Diluent Blank**	0.29	0
B1, B2	12.5 U/ml **Calibrator**	0.58	12.5
C1, C2	25 U/ml **Calibrator**	0.83	25
D1, D2	50 U/ml **Calibrator**	1.30	50
E1, E2	100 U/ml **Calibrator**	2.42	100
G1, G2	**Sample** [Diluted 1:100]	1.32	51.0
	Calculated: 100-fold dilution x 50 U/ml = **5.0 kU**/ml in serum		



This is for demonstration purpose only. Use the curve that is generated with the test.

/6-ADI_ELISA_630-135

PRODUCT SPECIFICATIONS

Specificity

Purified MBP is used to coat the microwells; thus the assay is specific for antibodies directed to MBP. The anti-Human IgM-HRP conjugate reacts with Human IgM only; IgA, IgG or IgE class antibodies would not be measured above background signals. These may also be detected using conjugates specific for each isotype. ADI can provide anti-MBP kits for rats, mice, humans and monkeys (please see website).

Sensitivity

The MBP coating level is optimized to differentiate anti-MBP IgM from background (non-antibody) signal with Human serum samples diluted 1:100 or more.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the kit box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Anti-MBP Positive Control Part No. 630-122	Two (2) vials containing anti-MBP lyophilized in buffer with protein as stabilizer. Keep refrigerated when not in use.
Reconstitute 1 vial with **Working Sample Diluent**, according to the vial label, to provide a 100 U/ml Positive Control; this may be used to prepare a Calibrator curve, if dilutions of positive samples are parallel (see Limits of the Assay). Prepare 2-fold dilutions, as follows:	

Standard	+ Diluent =	Concn
Reconstituted Positive Control	None	100 U/ml
225 ul of 100 U/ml	225ul	50 U/ml
225 ul of 50 U/ml	225ul	25 U/ml
225 ul of 25 U/ml	225ul	12.5 U/ml

Use within 2 weeks of preparation; store at 2-8^{o} C.

Sample Diluent Concentrate (20x) Cat. No. SD-20TG, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8^{o}C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Human IgM - HRP Conjugate Concentrate (100x) Part No. ____, 0.15ml	Peroxidase conjugated anti-Human IgG in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8^{o}C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
MBP Microwell Strip Plate	630-101	8-well strips (12)	Coated with MBP, and post-coated with stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgM-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

Samples and Controls

Prepare 1:10 stock of sample in 1x sample diluent (10 ul sample and 90 ul diluent). The samples are stable in this buffer at 4oC for several weeks. Perform all subsequent testing from 1:10 stock (e.g 1:200 or 1:1000 etc). It will avoid freezing and thawing of sample. Dilute **Samples** in Working Sample Diluent according to expected anti-MBP activity levels; for serum: dilute at least 200-fold (e.g., 5ul sample + 995 ul Diluent) for reduced nonspecific signals. At least 2 dilutions of each sample is recommended in order to determine if reading values from the Calibrator curve is valid (see Limits of the Assay).

Include Working Sample Diluent as a Diluent Blank to determine proper assay performance (signal should be < 0.4 OD) and to subtract from sample and standard values to obtain net OD. Internal **Controls** that represent the lab's expected results should also be included in each assay run.

Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 2 wells for each Sample and Control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

ASSAY PROCEDURE

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human Ig-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.