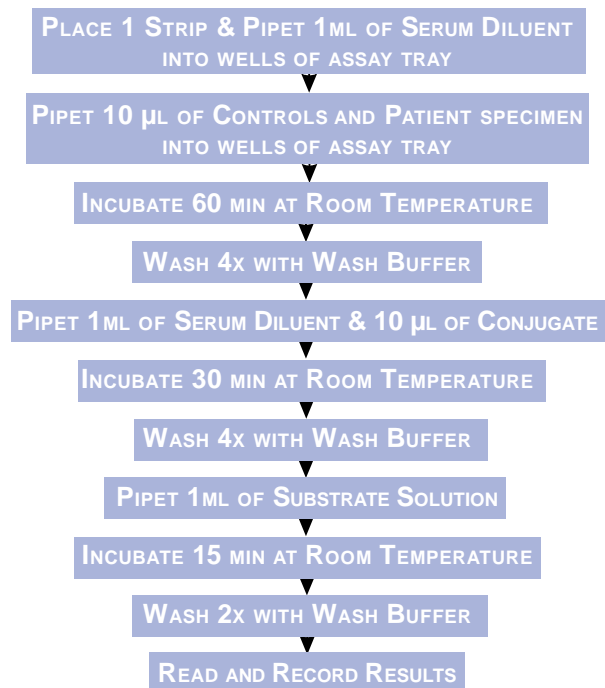


## ImmuBlot™ PROCEDURE AT A GLANCE



For technical assistance please contact:



**IMMCO Diagnostics, Inc.**  
60 Pineview Drive  
Buffalo, NY 14228-2120  
Telephone: (716) 691-0091  
Fax: (716) 691-0466  
Toll Free USA/Canada: 1-800-537-TEST  
E-Mail: [info@immcodiagnostics.com](mailto:info@immcodiagnostics.com)

or your local product distributor



## ImmuBlot™ Anti-Myelin Associated Glycoproteins (anti-MAG) Western Blot Immunoassay Research Use only

### PRODUCT INSERT

Code: 1173

20 Determinations

### INTENDED USE

A Western Blot Immunoassay for the detection of antibodies to primarily anti-myelin associated glycoproteins (anti-MAG) and other glycolipid autoantibodies in human serum.

### SUMMARY AND EXPLANATION

Autoimmune responses of the peripheral nervous system, recognized as *peripheral neuropathies*, are manifestations associated with autoantibodies against various neural glycoconjugates. These neuropathies can be acute, chronic, involve axonal degeneration, or demyelination. Autoimmune neuropathies can be further divided into monoclonal gammopathies and polyclonal inflammatory polyneuropathies like Guillain-Barré syndrome, *Chronic Inflammatory Demyelinated Polyneuropathy* (CIDP), *Multifocal Motor Neuropathy* (MMN), and *paraneoplastic neuropathies*. In these diseases, there is a significant overlap of the involved auto-antigens which mediate the pathogenic mechanism. The following peripheral nerve specific autoantibodies are found in these neuropathies<sup>1-6</sup>:

- a) **anti-Myelin associated glycoprotein (MAG),**
- b) **anti-acidic glycolipids like sulfoglucoronyl paragloboside (SGPG),**
- c) **anti-gangliosides**
- d) **anti-compact myelin associated proteins like P0, P2, and peripheral myelin protein 22 (PMP22).**

The epitope (HNK1) recognized by the human MAG autoantibodies is a sulfated oligosaccharide. This same epitope is shared by SGPG, P0 and PMP22<sup>7</sup>. Neuropathies associated with anti-MAG with IgM paraproteinemia are usually a heterogeneous disease group, slowly progressive with evidence of demyelination and a variable degree of axonal loss usually associated with gait ataxia. Of all peripheral neuropathy cases with IgM paraproteinemia, 50% possess anti-MAG antibodies<sup>8</sup>. It is perceived that these autoantibodies might interfere with the process of myelination, with myelin maintenance, or with axon-Schwann cell interactions. Hence, the detection of these autoantibodies is useful for the clinician, as it suggests active demyelination in a peripheral neuropathy.

The Western Blot immunoassay provides a sensitive method for the simultaneous screening and confirmation of autoantibodies against various nerve myelin associated antigens. Anti-MAG reactions can easily be observed at 100 kD. If the specimen yields no immunoreactivity on the blot strip, the result should be reported as negative.

## PRINCIPLES OF PROCEDURE

To perform the test, strips are incubated with diluted patient serum. Antibodies specifically bind to the myelin associated antigens on the strip. After proper washing and an incubation step with goat anti-human IgM conjugate, strips are washed and incubated with enzyme substrate. Anti-MAG antibody positive reactions appear as blue-violet bands at 100 kD.

## REAGENTS

### Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.** Do not use, if liquid reagents are turbid or a precipitate is present. Prior to starting the assay, reagents must be equilibrated to room temperature (~22°). Antigen strips can only be used once. Do not interchange components of different lots. Do not use reagents beyond expiration date indicated on labels.

### Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by US FDA required tests. However, all human blood derivatives and patient specimens should be considered potentially infectious and good laboratory practices in storing, dispensing and disposing of these materials must be followed.

**WARNING** - Sodium azide ( $\text{NaN}_3$ ) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup.  $\text{NaN}_3$  is toxic if ingested. Report incidents immediately to laboratory director or poison control center.

Follow good laboratory practices to minimize microbial and cross contamination of reagents.

### Materials provided

ImmuBlot™ anti-MAG Western Blot

Product Code: 1173

Kit contains sufficient reagents to perform 20 determinations.

<b>1 x 20</b>	<b>Western Blot Strips</b>
<b>1 x 110 µl</b>	<b>*anti-MAG Positive Control</b> (purple vial cap)
<b>1 x 110 µl</b>	<b>*Negative Control</b> (yellow vial cap).
<b>1</b>	<b>Control Card</b>
<b>1 x 240 µl</b>	<b>*goat anti-human IgM Conjugate</b> (blue vial cap)
<b>1 x 60 ml</b>	<b>*Serum Diluent</b>
<b>1 x 25 ml</b>	<b>Enzyme Substrate</b> (amber bottle)
<b>1 vial</b>	<b>*Powdered Wash Buffer</b> ; reconstitute to one liter with deionized or distilled water.
<b>3</b>	<b>Assay trays</b>
<b>2</b>	<b>Report Forms</b>

\*Contains <0.1%  $\text{NaN}_3$

## LIMITATIONS OF THE PROCEDURE

The ImmuBlot™ anti-MAG antibody Western Blot should be used as an aid to diagnosis. Positive results may be found in other autoimmune conditions and/or certain infectious diseases. Hence results should be evaluated and interpreted by the clinician or neurologist in light of the patient's clinical history and other laboratory findings. Some sera may react to the MW marker occasionally, the significance of which is not known.

## TROUBLESHOOTING GUIDE

- **Strong band/s on Negative Control strip.** Likely cause: contaminated Negative Control vial, or cross contamination from well containing a positive serum.
- **Positive Control appears like Negative Control strip.** Likely cause: Negative Control vial was confused as Positive Control vial.
- **Strips are completely blank.** Likely cause: addition of the Conjugate or Substrate was omitted.
- **High background and poor contrast between bands and background.** Likely cause: wash step(s) may have been omitted or incorrectly performed, or incubations were overextended.

## REFERENCES

1. Quarles RH, and Weiss MD. Autoantibodies associated with peripheral neuropathy. *Muscle & Nerve*; 22:800-822, 1999.
2. Griffin J. Antigliocolipid antibodies and peripheral neuropathies: links to pathogenesis. *Prog Brain Res*; 101: 313-323, 1994.
3. Quarles RH. Glycoproteins of the myelin sheaths. *J. Mol. Neurosci*; 8:1-12, 1997.
4. Kanda T, Yoshino H, Ariga T et al. Glycosphingolipid antigens in cultured bovine brain microvascular endothelial cells: Sulfoglucuronosyl paragloboside as a target of monoclonal IgM in demyelinating neuropathy. *J. Cell Biol*; 126:235-246, 1994.
5. Hammer JA, O'Shannessy DJ, and De LM et. al. Immunoreactivity of PMP-22, P0, and other 19 to 28kDa glycoproteins in peripheral nerve myelin of mammals and fish with HNK1 and related antibodies. *J. Neurosci Res*; 35:546-558, 1993.
6. Baba H, Daune GC, Ilyas AA et. al. anti-GM1 ganglioside antibodies with differing fine specificities in patients with multifocal motor neuropathy. *J. Neuroimmunol*; 25:143-150, 1989.
7. Field MC, Wing DR, Dwek RA et. al. Detection of multisulfated N-linked glycans in the L2/HNK1 carbohydrate epitope expressing neural adhesion molecule P0. *J. Neurochem*; 58:993-1000, 1992.
8. Latov N. Pathogenesis and therapy of neuropathies associated with monoclonal gammopathies. *Ann. Neurol*; 37:S32-S42, 1995.
9. Biosafety in Microbiological and Biomedical Laboratories. Center for Disease Control, National Institute for Health. (HHS Pub. No {CDC} 93-8395) 1993.
10. Chassande B, Leger JM, Younes-Chennoufi AB et al. Peripheral neuropathy associated with IgM monoclonal gammopathy: Correlations between M-Protein antibody activity and clinical/electrophysiological features in 40 cases. *Mus and Nerve*; 55-62, 1998.
11. Meucci M, Baldini L, Cappellari A et al. Anti-Myelin associated glycoprotein antibodies predict the development of neuropathy in asymptomatic patients with IgM monoclonal gammopathy. *Ann Neurol*; 46:119-122, 1999.

## EXPECTED VALUES

### Incidence of anti-Nerve (anti-MAG) Antibodies in Neuropathies<sup>10</sup>

Disorder	n	positive	% positive
Polyneuropathy+ IgM gammopathy	40	26	65
Demyelinating neuropathies	33	26	78
Axonal neuropathy	6	0	0

### Association of anti-MAG Antibody Titers with Progression to Clinical Neuropathy<sup>11</sup>

anti-MAG Titer	no neuropathy n=17	subclinical n=7	confirmed clinical n=6
High 1:25,000,000 to 1:100,000	1	3	3
Low 1:6,400 to 1:200	6	2	1
Negative <1:10	10	2	2

## Materials Required But Not Provided

- Clean 1000 ml graduated cylinder
- Non-serrated forceps (Filter forceps)
- Rocker or rotating platform shaker
- Absorbent paper or paper towels
- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettes capable of delivering 10 to 1000 µl
- Disposable pipet tips
- Timer

## SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

## PROCEDURE

### Procedural Notes

- Read Product Insert carefully before starting with the assay.
- Let serum specimens and test reagents equilibrate to room temperature for ~30 minutes prior to starting the test procedure. Return all unused specimens and reagents to the refrigerator promptly after use.
- Proper washing technique is critical to the satisfactory performance of the assay.
- Manipulate test strips with clean forceps only. Do not touch with bare hands.
- Strips are individually numbered at the bottom of each strip. Assign specimen identification numbers to the respective strips on the Report Form.
- Complete all other relevant information on the Report Form prior to starting the assay.

### Test Method

- Step 1** Using blunt forceps, place required number of **Strips** labeled side up into individual wells of the assay tray.
- Step 2** Pipet **1.0 ml** of Serum Diluent into each well.
- Step 3** Pipet **10 µl** of Positive and Negative Control and patient sample into appropriate wells to obtain a **1:101 dilution**. Incubate **60 minutes** (±5 min.) at room temperature on a rocker or rotating shaker.
- Step 4** Aspirate sample solution into waste container. Thoroughly wash strips with Wash Buffer by squirting approximately 2ml of solution directly onto strips. Wash strips with gentle agitation for **5 minutes** and aspirate solution into waste container. **Repeat 3x**. *Caution: Complete washing of the strips between incubations is crucial to obtain valid results. Improper washing will result in high background staining.*

- Step 5** Pipet **1.0 ml** of Serum Diluent followed by **10 µl** of Conjugate into each well. Incubate **30 minutes** ( $\pm 5$  min) at room temperature on rocker or rotating shaker.
- Step 6** Repeat **Step 4**.
- Step 7** Pipet **1.0 ml** Substrate into each well and incubate with gentle shaking **15 minutes** ( $\pm 5$  min) at room temperature and reduced light.
- Step 8** Repeat **Step 4**, washing twice instead of four times.
- Step 9** Using blunt forceps, remove strips from assay tray and place them gently onto absorbent paper. Handle strips only at the ends and let them dry **15-20 minutes**.

### Quality Control

Though the control cards are lot specific, negative and positive controls must be included in each test run to ensure proper performance of the assay.

*Positive Control Reaction:* A dense and diffused blue-violet band will appear at 100 kD, representing the anti-MAG reaction. In addition the positive control strip will exhibit a blue 35 kD alignment marker (refer to Figure 1).

*Negative Control Reaction:* The negative control reaction will only exhibit the blue-violet 35 kD alignment marker (refer to Figure 2)

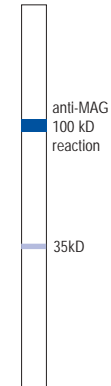
## RESULTS

### Reading and Interpretation Guidelines

The ImmuBlot™ strips contain myelin associated glycoproteins of 100 kD molecular weight. The 35 kD protein serves as a molecular weight marker to help align the strips on the control card (refer to Figure 1).

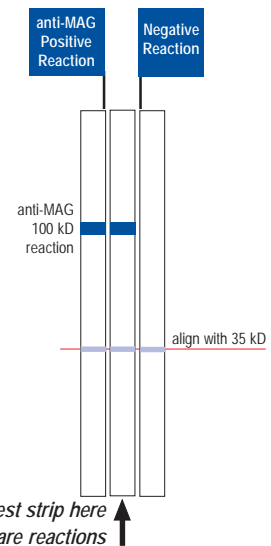
- Step 1** Hold test specimen strip between the positive and negative control strips on the provided, laminated Control Card and align test strip using the 35 kD molecular weight marker as the reference point (refer to Figure 2).
- Step 2** Compare reaction of test strip with those of the controls on either side.

**Figure 1**



**Figure 2**

ImmuBlot™ anti-MAG ab.  
Western Blot



- Step 3** If the band on the test strip aligns with the 100 kD band on the positive control strip it is an anti-MAG reaction. Such a reaction should be considered positive. Positive reactions can also occur in varying intensities, from weak to strong. Weak reactions should be compared with baseline reaction intensities at the corresponding position on the negative control strip. Figure 2 provides an example of a properly aligned positive result.
- Step 4** If there are no reaction bands visible and/or bands not corresponding to the 100 kD anti-MAG reaction of the control card these results should be considered anti-MAG negative.