INTENDED USE

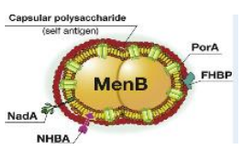
The **Mouse anti-Meningococcal serogroup B (Men-B) Neisserial adhesin A (NadA) IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for Nada antigen in serum or plasma of vaccinated, immunized and/or infected hosts. This kit employs recombinant Men-B proteins (**Nada**). This immunoassay is suitable for:

- Determining **immune status** of Mouse;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing Qualifying and standardizing vaccine batches & protocols.

The kit has no live or killed bacteria or virus. It uses recombinant purified antigens. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

GENERAL INFORMATION

Meningococcal meningitis, a form of meningococcal disease, which is a serious bacterial infection, is caused by bacteria called **Nisseria meningitidis also known as meningococcus**. N. meningitidis has 13 clinically significant serogroup classified according to the antigenic structure of their polysaccharide capsule. Six serogroup, A, B, C, Y, W135 and X are responsible for virtually all cases of the disease in humans. Disease caused by N. meningitides remains a major worldwide cause of morbidity and mortality, even after the development of vaccines to protect against several meningococcal serogroup.



The **Men B vaccine** contains the genome derived antigens **GNA2132** (Neisserial Heparin Binding Antigen, or **NHBA**), **GNA1870** (factor H binding protein, or **NadA)** and **GNA1994** (Neisseria adhesin A or **NadA**). Two additional antigens, **GNA2091** and **GNA1030**, were also selected because they induced protective immunity but only in some of the assays. The antigens were combined in a **multi component vaccine** with the aim of inducing better and broader protection.

PRINCIPLE OF THE TEST

The Mouse Anti- **Men-B NadA IgG** ELISA kit is based on the binding of IgG in samples to the antigen coated on the microwells, Bound antibody is detected by antibody-IgG-HRP conjugate. After a washing step, substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of IgG present in the sample. Stopping Solution is added to terminate the reaction and convert blue to yellow color. Absorbance is measured at 450nm using an ELISA reader. The presence of Mouse anti-**Nada IgG** antibody in samples is determined relative to Calibrators.

KIT CONTENTS

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

To Be Reconstituted: Store as indicated.

.	Preparation Instructions
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 4°C for long term and RT for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** (**WSD**) and store at 2-8°C until the kit lot expires or is used up.
Anti-Mouse IgG-HRP Conjugate Concentrate (100x) Part: H-MsG.211 , 0.15ml	in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Men-B NadA Coated Strip Plate	600906	8-well strips (12)	Coated with purified NadA protein and post-coated with stabilizers.
Anti-Men-B NadA IgG Calibrators			
3 U/ml 10 U/ml 30 U/ml 90 U/ml	600907A 600907B 600907C 600907D	1 ml 1 ml 1 ml 1 ml	Four (4) vials, each containing anti-NadA proteins in a buffer with antimicrobial.
Mouse Anti-NadA IgG Positive Control	600908PC	1 ml	Mouse anti-NadA IgG diluted in buffer with protein, detergents and antimicrobial. Net OD > 0.5
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Diluted sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- ELISA reader at 450 nm and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

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, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Caution: Mouse serum and other bodily fluids may contain infectious material. Always wear gloves when handling Mouse samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Sample Dilution & Antibody Stability

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further test dilutions (1:100 or higher) should be made from the initial stock and use the same day as the assay. Do not store test dilutions beyond the assay date.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]

Further (1/100): **10**ul initial (1/5)+1**90**ul WSD (1/100)

We recommend initial testing of samples **at 1:100** dilution. Samples can be diluted further to bring a450 to within the range of the test..

Assay Design

Review Interpretation of Results and Limits of the Assay (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **3 U/ml Calibrator**. This is usually 1/100 or greater dilution for Mouse serum/plasma with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the Mouse Anti-Meningococcal IgG **Positive Control**; net OD > **0.5**.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **90 U/ml** should give a high signal (>1.0 OD); **10 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

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 8 Calibrator wells and 2 wells for each sample 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

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

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

- Add 100ul of 1x sample diluent (blank), 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-Mouse IgG HRP 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 commercial 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 15 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

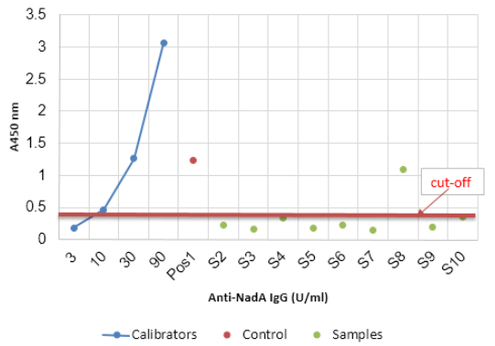
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **10 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



1-nas//600-905-MNG-ELISA-Graph1

Results

The **sensitivity** of the assay to detect anti-Men-B IgG, from either natural infection or vaccination, is controlled so that the **10 U/ml Calibrator** represents a threshold OD for most true positives in Mouse serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-Men-B antiserum, derived from Men-B vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
10 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – a Mouse antibody positive for Men-B different from Calibrators; net OD > **0.5**.

Internal Control – a true positive from a normal individual that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the 10 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples– 9 samples (S2-7, S9-S10) are negative: below the threshold; 1 sample (S8) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

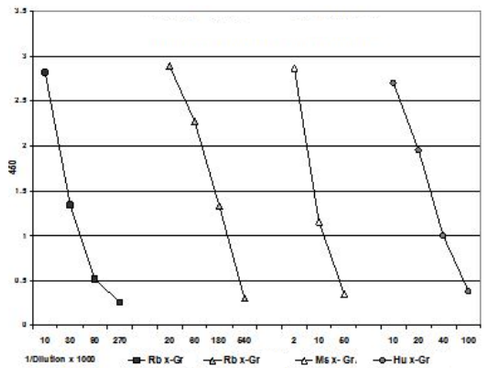
INTERPRETATION OF RESULTS (cont)

- Divide each Sample net OD by the 10 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

B. Antibody Titer from the standard graphs

The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading midrange in the dilution curves of each antibody as **Index.** In the example below, titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay. Rabbits were vaccinated with various individual proteins of Men-B vaccines and tested in the test.



Results

Rb x-Gr A: rabbit immunized with the vaccine A. Titer: **47.5 k**
Rb x-Gr B: rabbit immunized with the vaccine B. Titer: **255 k**
Rb x-Gr C: antibody specific to Vaccine C . Titer: **13.6 k**
Rb x-Gr D: antibody to Vaccine D Titer: **40 k**

The data shows that antibodies to 4 vaccine formulations of Besero Men-B vaccine produce antibody titers that varies from one formulation to another.

Assay Sensitivity

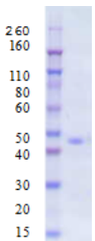
The Men-B NadA antigen coating level, HRP conjugate concentration and Sample Dilutions are optimized to differentiate anti-Men-B NadA protein's IgG from background (non-antibody) signal with Mouse serum or plasma **samples diluted 1:100** or more. Users may opt to use different dilution for testing vaccinated sample based upon antibody levels.

Limits of the Assay

- The assay detects and quantifies IgG antibodies directed to the Men-B NADA protein. Mouse or animals vaccinated or exposed to Men-B may not produce antibodies specific to NADA. We recommend testing of samples using other Men-B antigens or using alternate detection methods.

- Anti-NADA antibody levels of an immunized animal may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.

Antigen Specificity



Highly purified recombinant meningitis B NadA protein (full length, ~36 kda, >95%, his-tag) was used as antigen. NadA protein/ adhesin/invasin, [Neisseria meningitides MC58/NM477/NM3173] is quite conserved in various B meningitis strains: The NadA/lipoprotein sequence conservations is 86-100% in various strains.

Antibody and animal specificity

Recombinant purified Men-B NadA protein is used to coat the microwells. Therefore, only antibodies to Men-B NadA are detected in the test. The anti-Mouse IgG HRP conjugate reacts specifically with Mouse IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals. ADI has other kits to detect antibodies to NadA and other Men-B antigens in Mouse, monkey, mouse, and rabbit.

PRECAUTIONS AND SAFETY INSTRUCTIONS (SDS)

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

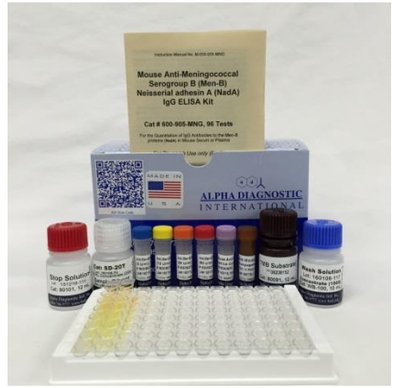
Instruction Manual No. M-600-905-MNG

Mouse Anti-Meningococcal Serogroup B (Men-B) Neisserial adhesin A (NadA) IgG ELISA Kit

Cat # 600-905-MNG, 96 Tests

For the Quantitation of IgG Antibodies to the Men-B proteins (**NadA**) in Mouse Serum or Plasma

For Research Use only (RUO)



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
Website: www.4adi.com