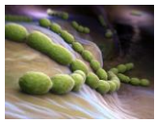
INTENDED USE

The Human Anti-Pneumococcal CPS13 IgM ELISA Kit quantifies IgM antibodies against capsular polysaccharides (CPS) of 13 serotypes (Pneumovax vaccine) in human serum/plasma of vaccinated or normal hosts. This immunoassay is suitable for:

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

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

GENERAL INFORMATION



Streptococcus pneumoniae, or pneumococcus, a Gram-positive bacteria, is one of the most common causes, along with *N. meningitides*, of bacterial meningitis in adults and young adults,Pneumococcal strains have capsular polysaccharides (**CPS**) that act as a virulence factor for the organism; more than 90 different serotypes are known. Serotype specific antibodies against the CPS provide protection against the corresponding strains. The pneumococcal vaccine most commonly used today consists of purified polysaccharides from **23 serotypes** (non-conjugated: **Pneumovax** by Merck).Pneumococcal conjugate vaccines (PCV) contain polysaccharides conjugated to diphtheria toxin **CRM197**, with three PCV vaccines currently available:**Prevnar-7** or PCV-7 (Wyeth) is a 7-valent vaccine;**Synflorix** (GSK) is a 10-valent vaccine (PCV-10);**Prevnar-13**(Pfizer) is 13-valent (PCV-13: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F); each are conjugated to carrier protein CRM197.

PRINCIPLE OF THE TEST

The Human Anti-CPS13 IgM ELISA kit is based on the binding of anti-CPS IgM in samples to the purified CPS antigens coated on the microwells; bound antibodies are detected by specific anti-human IgM-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed, which is directly proportional to the amount of antibody present in the samples. Stopping Solution is added to terminate the reaction, and absorbance at 450nm (yellow color) is then measured using an ELISA reader. The activity of anti-CPS IgM in samples is determined relative to anti-CPS specific Calibrators.

PRODUCT SPECIFICATIONS

Specificity

The plate is coated with a mixture of 13 purified non-conjugated CPS (1, 2, 3, 4, 5, 6B, 7F, 8,9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F); no antibodies to CRM197 will be detected. These CPS preparations are contaminated with **CWPS** (Cell Wall PolySaccharide), which is common to all pneumococci, both virulent and avirulent strains. An **adsorption step** may be required to remove these antibodies prior to testing for antibodies to the CPS. The WHO has recommended an extra absorption step with **22F CPS** along with **CWPS** to effect a better measurement of CPS-specific antibodies. **ADI provides a separate kit to perform and measure CWPS/22F absorption (#560-410-C22) as well as the absorbent reagent (#560-CW-Abs)**

The anti-Human IgM HRP conjugate specifically detects IgM, and does not react with IgG, IgA or IgE class antibodies. Other ELISA kits are available from ADI to detect antibodies to individual CPS (e.g., 6B).

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C 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.

Component	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-Human IgM-HRP Conjugate Concentrate (100x) Part: H-HuM.211, 0.15ml	Peroxidase conjugated anti-human IgM 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
Pneumococcal 13 Type CPS Coated Strip Plate	560-13	8-well strips (12)	Coated with 13 serotypes of CPS antigen,
Anti-Pneumococcal Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	560-07rSB 560-07rSC 560-07rSD 560-07rSE	0.65ml 0.65ml 0.65ml 0.65ml	Four (4) vials, each containing anti-pneumococcal CPS; in buffer with antimicrobial.
Human Anti-Pneumococ-cal IgM Positive Control	560-071323-PCM	0.65ml	Human anti-CPS IgM; diluted in buffer with protein, detergents and antimicrobial. Net OD >**0.5**
Low NSB Sample Diluent (LNSB) Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes
- 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. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Sample Stability & Dilution

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 dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design

Review Interpretation of Results (p5-7) before proceeding:

- Pre-absorb samples with **CWPS/22F** antigen, if necessary (see Specificity, page 1)
- 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 **1 U/ml Calibrator**. This is usually 1/100 or greater dilution for human serum 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 Human Anti-Pneumococcal IgM **Positive Control**; net OD >**0.5**.
- Runa set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 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 8Calibrator 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 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 IgM 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 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.

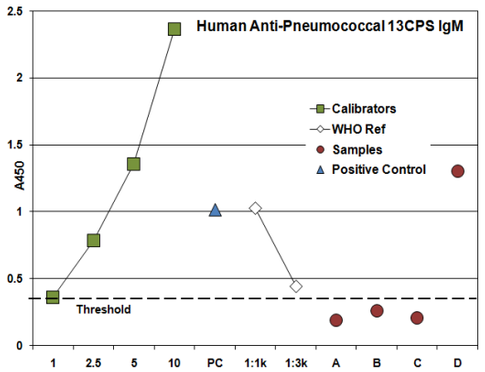
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

=Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-Pn 13CPS IgM, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:250 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-Pn 13CPS antiserum, derived from Pn CPS vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 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 – human serum with reactivity to Pn 13CPS; net OD > 0.5. This Control can be used to normalize between-assay variation.

WHO Reference Serum – a pooled serum of individuals vaccinated with Pneumococcal 23CPS (Pneumovax II; Merck; NIBSC code 007sp). **Assay Sensitivity:** the **2.5** U/ml **Calibrator** = **1/1.6k** dilution of the **WHO Reference** (CWPS/22F-absorbed)**.**

Samples A,B,C,D – 3 samples (1/250) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

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

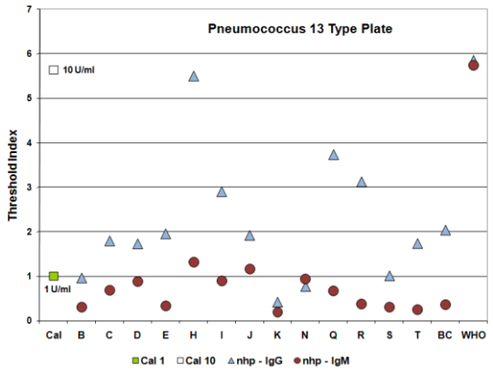
- ❖ Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Normal Human Serum/Plasma (NHS/P), **Absorbed**

A panel of human serum/plasma of unknown history, and the WHO Reference 007sp, were absorbed with **CWPS/22F** (see page 3) and tested for anti-CPS13 IgG (1/1k dilution) and IgM (1/250 dilution). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti-Pn IgG: most samples were positive at 1/1k dilution; one serum and the WHO Reference were highly positive.

Anti-Pn IgM: most samples were negative or borderline at 1/250 dilution; the WHO Reference was highly positive.

Notes:

- Positives may be due to prior vaccination and/or exposure to Pneumococcal bacteria.
- The CWPS/22F-absorbed sera can be assayed on plates coated with individual Pn CPS to serotype the positives.

B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

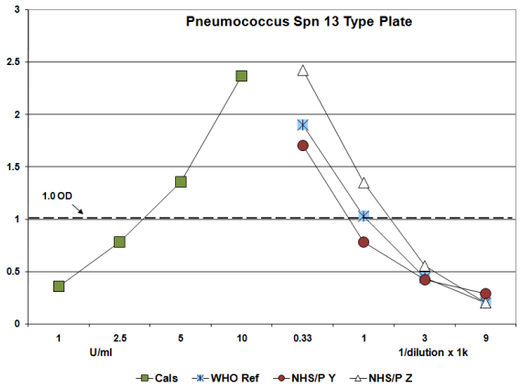
1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

INTERPRETATION OF RESULTS (cont)

C. Antibody Titer
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, **IgM** titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.



Results

Calibrators: Titer: **3.3 U/**ml. The Calibrator titer value can be used to normalize between-assay sample titer values.

WHO Reference 007Sp: a high titer serum pool of individual humans immunized with Pneumococcal 23 CPS-valent vaccine (Pneumovax; Merck). Titer: **1.1 k**

NHS/P Y: a normal human plasma of unknown history.
Titer: **0.7 k**

NHS/P Z: a normal human plasma of unknown history.
Titer: **1.6 k**

PRECAUTIONS AND SAFETY INSTRUCTIONS

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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

Instruction Manual No. M-560-175-13M

Human Anti-Pneumococcal CPS13 IgM ELISA Kit

Cat. # 560-175-13M, 96 tests

For Quantitation of Anti-CPS IgM to 13 Serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A, and 23F) in Human Serum or Plasma

For research use only, not for diagnostic or therapeutic use.

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Note: Most human sera (vaccinated or normal) have non-specific antibodies against CWPS/22F sufficient to interfere with specific CPS antibody testing. These CWPS/22F-specific antibodies should be removed by absorbing with CWPS/22F using an ADI kit (#560-410-C22) or in-house or ADI absorbent (#560-CW-Abs). Please contact ADI.