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VaccZyme™ Anti-PCP IgG Enzyme Immunoassay Kit

For *in vitro* diagnostic use

Product code: MK012

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1 INTENDED USE

This assay is designed for the *in vitro* measurement of specific IgG antibodies against Pneumococcal Capsular Polysaccharide (PCP) present in human serum.

Sufficient materials are supplied to allow a maximum of 41 samples to be tested in duplicate or 89 in single, with a calibration curve and two controls.

2 BACKGROUND

This kit is designed to measure antibody responses to pneumococcal vaccines incorporating 23 polysaccharides isolated from *Streptococcus pneumoniae*.¹ These polysaccharides represent approximately 80% of the commonly encountered virulent serotypes. Immunisation with 23-valent vaccines is recommended for elderly patients to reduce death due to pneumonia and for children over 2 years that are at risk of severe life threatening pneumococcal infection.²

The response in 30% of subjects to vaccination with *S. pneumoniae* is attributable to C-polysaccharide (CPS) antibodies and not to specific anti-PCP IgG. These CPS antibodies confer limited protection against pneumococcal infection; consequently CPS absorption has been incorporated in this assay.³

A patient's antibody response may be evaluated by the serological determination of their IgG anti-PCP antibody levels pre- and post-vaccination using this quantitative enzyme immunoassay. Patients with repeated bacterial infections should be investigated for immunodeficiency and thus inability to respond to specific polysaccharide antigens (review^{4,5}).

3 PRINCIPLE OF THE ASSAY

Microwells are pre-coated with the PCP antigen (1-5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F – Danish nomenclature). Calibrators and controls are pre-adsorbed against capsular polysaccharide and samples are diluted in a diluent containing CPS. The calibrators, controls and diluted patient samples are added to the wells and antibodies recognising the PCP antigen bind during the first incubation.

After washing the wells to remove all unbound proteins, purified peroxidase labelled rabbit anti-human IgG (γ chain specific) conjugate is added. The conjugate binds to the captured human antibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualised with 3,3',5,5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of antibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point colour, which is read at 450nm.

4 PRECAUTIONS

4.1 WARNING

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

This product contains sodium azide and ProClin™ 300 and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

The buffers and serum supplied in this kit contain various enzyme inhibitors as listed below. These are hazardous and should be handled with care.

INHIBITOR	CONCENTRATION
Sodium Azide	0.099%
ProClin 300	0.02%-0.045%
Bromonitrodioxane	0.002%
Methylisothiazone	0.002%

The stop solution contains 3M phosphoric acid, which is an irritant. Avoid contact with skin or eyes.

Reagent spills should be cleaned up appropriately, observing local and environmental regulations.

4.2 CAUTION

This product should only be used by appropriately trained personnel. Strict adherence to the protocol is recommended. Any deviation may affect assay performance, and the results obtained. Pay attention to specific 'Notes' and warnings throughout these Instructions for Use.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all reagents are from the **SAME**

batch. All strips must be taken from the same foil pouch. Substitution of any component may lead to incorrect results.

To avoid reagent contamination, especially of the conjugate and calibrators, only use new or clean plastic/glassware. NEVER return unused reagents to the bottles.

Do not leave reagent bottles uncapped; any resulting evaporation or contamination will lead to inconsistent results.

TMB substrate must not be exposed to light or water.

Microbially contaminated, haemolysed or lipaemic serum and specimens containing particulate matter should not be used.

Inaccurate sample dilution cannot be checked, as kit controls are ready to use. The use of calibrated pipettes and appropriate internal QC samples is recommended.

When using automated assay systems, sample dilutors and other automated equipment, follow the manufacturer's instructions carefully. Particular attention should be given to the setup of the equipment and installation and connection to external services. All settings for automatic washers and readers must be followed carefully and equipment must be maintained and serviced according to the manufacturer's instructions.

4.3 STORAGE AND STABILITY

The kit should be stored at 2-8°C and should **not** be frozen. Inappropriate storage temperatures will affect the results.

Wash buffer diluted into a clean container can be stored at room temperature for a maximum of 4 weeks.

The expiry date of the kit is shown on the outer label.

5 SAMPLE COLLECTION AND STORAGE

Blood samples should be collected by venepuncture allowed to clot naturally and the serum separated.

The serum may be stored at 2-8°C for up to 48 hours prior to assay, or for prolonged storage kept undiluted at -20°C or below.

Repeated thawing and freezing should be avoided.

Serum samples should **not** be heat-inactivated, as this may give false positive results.

6 MATERIALS

6.1 MATERIALS SUPPLIED

- 6.1.1 Instruction Leaflet: Giving full assay details.
- 6.1.2 QC Certificate: Indicating the expected performance of the batch.
- 6.1.3 *PCP Coated Wells*: 12 breakapart 8 well strips coated with PCP antigen. Each plate is packaged in a re-sealable foil bag containing two desiccant pouches.
- 6.1.4 *PCP IgG Sample Diluent*: 2 bottles containing 50mL of CPS buffer for sample dilution. Coloured yellow, ready to use.
- 6.1.5 *Wash Buffer*: 1 bottle containing 50mL of a 20-fold concentrated buffer for washing the wells.
- 6.1.6 *PCP IgG Calibrators*: 5 bottles each containing 1.2mL of diluted human serum, with the following concentrations of anti-PCP antibody: 270, 90, 30, 10, 3.3mg/L. Ready to use.
- 6.1.7 *PCP IgG High Control*: 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- 6.1.8 *PCP IgG Low Control*: 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- 6.1.9 *PCP IgG Conjugate*: 1 bottle containing 12mL of purified peroxidase labelled antibody. Coloured red. Ready to use.
- 6.1.10 *TMB Substrate*: 1 bottle containing 14mL TMB substrate. Ready to use.
- 6.1.11 *Stop Solution*: 1 bottle containing 14mL of 3M phosphoric acid. Ready to use.

6.2 ADDITIONAL MATERIALS AND EQUIPMENT - not supplied

- 6.2.1 Automatic microplate plate washer: This is recommended, however, plate washing can be performed manually.
- 6.2.2 Plate reader: Capable of measuring optical densities at 450nm referenced on air.
- 6.2.3 Distilled or deionised water: This should be of the highest quality available.
- 6.2.4 Calibrated micropipettes: for dispensing 1000, 100 & 10µL.
- 6.2.5 Multichannel pipette: Recommended for dispensing 100µL volumes of conjugate, substrate and stop solution.
- 6.2.6 Glass/plastic tubes: For sample dilution.

7 ASSAY METHOD

7.1 PRE-ASSAY STEPS

1. Bring the kit to room temperature

The kit is designed for room temperature operation (20-24°C). Remove the kit from storage and stand at room temperature for approximately 60 minutes.

Wells must **not** be removed from the foil bag until they have reached room temperature.

Note: The kits may be maintained at room temperature for up to 1 week.

2. Kit components

Gently mix each kit component before use.

3. Wash buffer dilution

Add 50mL of the wash buffer concentrate to 950mL of distilled water (1 in 20 dilution) into a clean container and mix. Smaller volumes can be diluted as appropriate.

Note: Diluted wash buffer can be stored at room temperature for up to 4 weeks, therefore only dilute the appropriate amount.

4. Sample dilution

Dilute 10µL of each sample with 1000µL of sample diluent and (1:100) and mix well.

Smaller volumes can be diluted as appropriate.

Note: Diluted sample **must** be used within 8 hours.

5. Strip and frame handling

Place the required number of wells in the strip holder. Position from well A1, filling columns from left to right across the plate. When handling the plate, squeeze the long edges of the frame to prevent the wells falling out.

Note: Return unused wells to the foil bag immediately with the two desiccant pouches and re-seal tightly to minimise exposure to moisture.

Take care not to puncture or tear the foil bag, see below.

WARNING: Exposure of wells to moisture or contamination by dust or other particulate matter will result in antigen degradation, leading to poor assay precision and potentially false results.

7.2 ASSAY METHOD

Maintain the same dispensing sequence throughout the assay.

1. Sample addition

Dispense 100µL of each calibrator, control and diluted (1:100) sample into the appropriate wells of the plate provided.

Note: Samples must be added to the plate as quickly as possible to minimise assay drift, and the timer started after the addition of the **last** sample.

Incubate at room temperature for 30 minutes.

2. Washing

The washing procedure is critical and requires special attention. An improperly washed plate will give inaccurate results, with poor precision and high backgrounds.

After incubation remove the plate and wash wells 3 times with 250-350µL wash buffer per well. Wash the plate either by using an automatic plate washer or manually as indicated below. After the final automated wash, invert the plate and tap the wells dry on absorbent paper.

Plates can be washed manually as follows:

- a. Flick out the contents of the plate into a sink.
- b. Tap the wells dry on absorbent paper.
- c. Fill each well with 250-350µL of wash buffer using a multichannel pipette.
- d. Gently shake the plate on a flat surface.
- e. Repeat a-d twice.
- f. Repeat a and b.

3. Conjugate addition

Dispense 100µL of conjugate into each well, blot the top of the wells with a tissue to remove any splashes.

Note: To avoid contamination, NEVER return excess conjugate to the reagent bottle.

Incubate at room temperature for 30 minutes.

4. Washing

Repeat step 2.

5. Substrate (TMB) addition

Dispense 100µL of TMB substrate into each well, blot the top of the wells with a tissue to remove any splashes.

Note: To avoid contamination never return excess TMB to the reagent bottle.

Incubate at room temperature in the dark for 30 minutes.

6. Stopping

Dispense 100µL of stop solution into each well. This causes a change in colour from blue to yellow.

7. Optical density measurement

Read the optical density (OD) of each well at 450nm on a microplate reader, within 30 minutes of stopping the reaction.

8 RESULTS AND QUALITY CONTROL

1. Quality control

In order for an assay to be valid, all the following criteria must be met:

- Calibrators and controls must be included in each run.
- The values obtained for the controls should be in the ranges specified on the QC Certificate.
- The curve shape should be similar to the calibration curve, shown on the QC Certificate.
If the above criteria are not met, the assay is invalid and the test should be repeated.

2. Calculate mean optical densities (for assays run in duplicate)

For each calibrator, control and sample calculate the mean OD of the duplicate readings. The percentage coefficient of variation (%C.V.) for each duplicate OD should be less than 15%.

3. Plot calibration curve

The calibration curve can be plotted either automatically or manually as follows by plotting the anti-PCP IgG antibody concentration on the log scale against the OD on the linear scale for each calibrator:

- Automatic - use appropriately validated software, and the curve fit that best fits the data.
- Manual - using log/linear graph paper, draw a smooth curve through the points (**not** a straight line or point to point).

4. Treatment of anomalous points

If any one point does not lie on the curve, it can be removed. If the absence of this point means that the curve has a shape dissimilar to that of the sample calibration curve, or more than one point appears to be anomalous, then the assay should be repeated.

5. Calculation of the control values

Read the level of the anti-PCP IgG antibody in the controls directly from the calibration curve. The values should fall within the ranges given on the QC Certificate.

6. Calculation of antibody levels in diluted samples

Read the level of the anti-PCP IgG antibody in the diluted samples directly from the calibration curve.

Note: The calibrator values have been adjusted by a factor of 100 to account for a 1:100 sample dilution. No further correction is required.

7. Assay calibration

The assay is calibrated against an affinity purified preparation of human antiserum to PCP.

8. Limitations

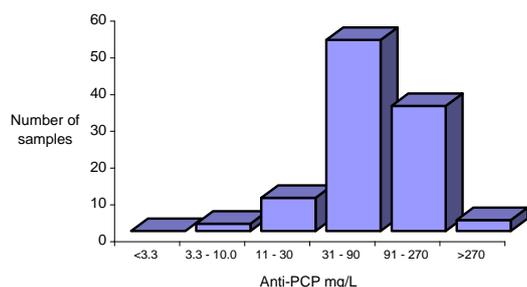
Pre- and post-vaccination samples should be run simultaneously.

This kit may be used to aid diagnosis of immunodeficiency only. Results must be confirmed by clinical findings and other serological tests.

The results obtained from this assay are not diagnostic proof of lack of protection / protection against pneumococcal infections, or of the presence or absence of immunodeficiency.

9 TYPICAL VALUES

Anti-PCP IgG antibodies levels were measured in serum from 100 normal adult blood donors (of unknown vaccination and immune status). The results displayed below are provided for illustration only and should not be used to calculate a normal range.



Antibody levels may vary according to vaccination history and immune status, as well as population group².

10 PERFORMANCE CHARACTERISTICS

10.1 PRECISION

The intra- and inter-assay precision were measured using three samples within the range of the calibration curve. The concentrations and % C.V. for each sample are given below:

INTRA-ASSAY PRECISION		
n=16	Concentration (mg/L)	% C.V.
Sample 1	10.7	5.2
Sample 2	42.5	3.2
Sample 3	172.9	6.1
INTER-ASSAY PRECISION		
n=3	Concentration (mg/L)	% C.V.
Sample 1	10.0	7.8
Sample 2	40.9	1.3
Sample 3	141.2	3.3

10.2 ANALYTICAL SENSITIVITY

Sensitivity was determined as the mean concentration + 2 SD given by 20 determinations of the sample diluent. This equates to 0.62mg/L.

10.3 MEASURING RANGE

The measuring range of the assay is 3.3 - 270mg/L.

10.4 INTERFERING SUBSTANCES

A range of interfering substances has been spiked into serum samples containing anti-PCP IgG antibody, which have then been subsequently assayed. The method used to check these substances was based on the Interference Check A plus™ kit - Kokusai Shiyaku, Japan.

Substance	Concentration	
Bilirubin F (Free)	180mg/dL	203mg/dL
Bilirubin C (Conjugate)	193mg/dL	202mg/dL
Haemolysed Haemoglobin	4820mg/dL	4860mg/dL
Chyle	14300 FTU	14600 FTU
Rheumatoid Factor	500 IU/mL	1035IU/mL

No interference was observed with free or conjugated bilirubin, haemoglobin, lipid or rheumatoid factor.

11 REFERENCES

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