ELISA kits available from ADI (see details at the web site)

Catalog#  Product Description

680-100-PCG  Mouse Anti-Phosphocholine (anti-PC) IgG ELISA Kit, 96 tests,
680-110-PCM  Mouse Anti-Phosphocholine (anti-PC) IgM ELISA Kit, 96 tests,
680-200-PCG  Rat Anti-Phosphocholine (anti-PC) IgG ELISA Kit, 96 tests,
680-210-PCM  Rat Anti-Phosphocholine (anti-PC) IgM ELISA Kit, 96 tests,
680-300-PCG  Human Anti-Phosphocholine (anti-PC) IgG ELISA Kit, 96 tests,
680-300-PCM  Human Anti-Phosphocholine (anti-PC) IgM ELISA Kit, 96 tests,
680-400-CCG  Mouse Anti-Cyclic Citrullinated Peptide (Anti-CCP) IgG ELISA Kit,
680-410-CCM  Mouse Anti-Cyclic Citrullinated Peptide (Anti-CCP) IgM ELISA Kit
680-420-CCG  Rat Anti-Cyclic Citrullinated Peptide (Anti-CCP) IgG ELISA Kit, 680-
430-CCM  Rat Anti-Cyclic Citrullinated Peptide (Anti-CCP) IgM ELISA Kit,
3300-205-APS  Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit,
3300-210-PSS  Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS  Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS  Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests,
3300-170-CLG  Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM  Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-195-B2M  Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative

CHECK LIST (Check each box after completing each step)

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
<th>Step 7</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KIT PROFILE

Date received:  Lot #  Exp.  
Date kit opened  Technician:  
Date used:  # Strips used  # remaining  
Date used:  # Strips used  # remaining  
Remarks  

Human Anti-Phosphocholine (PC) IgG ELISA KIT Cat. # 680-300-PCG

For Detecting Human IgG antibodies against Phosphocholine in Serum or Plasma

For In Vitro Research Use Only

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
Web Site:  www.4adi.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.
**Human Anti-Phosphocholine (PC) IgG ELISA Kit Cat. # 680-300-PCG (96 tests)**

<table>
<thead>
<tr>
<th>Kit Components (96 tests)</th>
<th>Cat #</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-antigen coated strip plate, (6x12 strip or 96 wells)</td>
<td>1 plate</td>
</tr>
<tr>
<td>Anti-PC IgG calibrator A (10 U/ml), 1 ml</td>
<td>1 vial</td>
</tr>
<tr>
<td>#680302A (white cap)</td>
<td></td>
</tr>
<tr>
<td>Anti-PC IgG calibrator B (25 U/ml), 1 ml</td>
<td>1 vial</td>
</tr>
<tr>
<td>#680302B (yellow cap)</td>
<td></td>
</tr>
<tr>
<td>Anti-PC IgG calibrator C (50 U/ml), 1 ml</td>
<td>1 vial</td>
</tr>
<tr>
<td>#680302C (green cap)</td>
<td></td>
</tr>
<tr>
<td>Anti-PC IgG calibrator D (100 U/ml), 1 ml</td>
<td>1 vial</td>
</tr>
<tr>
<td>#680302D (orange cap)</td>
<td></td>
</tr>
<tr>
<td>Anti-Human IgG Conjugate, 10X, (1.2 ml)</td>
<td>1 vial</td>
</tr>
<tr>
<td>#GAH1-1 (brown vial)</td>
<td></td>
</tr>
<tr>
<td>Sample/Conjugate Diluent (20X), 10 ml</td>
<td>1 bottle</td>
</tr>
<tr>
<td>#SD20T</td>
<td></td>
</tr>
<tr>
<td>Low NSB Diluent (green solution) 30 ml</td>
<td>1 bottle</td>
</tr>
<tr>
<td>#TBTm</td>
<td></td>
</tr>
<tr>
<td>Wash buffer (100X) 10 ml</td>
<td>1 bottle</td>
</tr>
<tr>
<td>#WB100 (blue cap)</td>
<td></td>
</tr>
<tr>
<td>TMB Substrate Solution, 12 ml</td>
<td>1 bottle</td>
</tr>
<tr>
<td>#80091 (brown bottle)</td>
<td></td>
</tr>
<tr>
<td>Stop Solution, 12 ml</td>
<td>1 bottle</td>
</tr>
<tr>
<td>#80101 (read cap)</td>
<td></td>
</tr>
<tr>
<td>Complete Instruction Manual; M-680-300-PCG</td>
<td>1</td>
</tr>
</tbody>
</table>

**Intended Use**

The Human Anti-Phosphocholine IgG (anti-PC IgG) ELISA Kit detects and quantifies phosphocholine-specific IgG in Human serum or plasma of normal or vaccinated individuals. This kit is for research use only, not for diagnostic procedures or therapeutic use.

**Introduction**

Phosphocholine (PC) is the hydrophilic polar head group of some phospholipids, which is composed of a negatively charged phosphate bonded to a small, positively charged choline group. PC has been detected on a number of pathogenic prokaryotes, including Streptococcus pneumoniae and other gram-positive bacteria such as other streptococci, Bacillus, Clostridium, as well as the gram-negative species H. influenzae. Anti-PC Ig's are naturally occurring autoantibodies that are created by CD5+/B-1 B cells and are referred to as non-pathogenic autoantibodies or innate antibodies. Induction of anti-PC Ig's is considered to be mainly due to exposure to S. pneumonia. Anti-PC Ig's that occur in atherosclerotic mice recognize the PC headgroup moiety of phosphatidylcholine in oxidized low density lipoprotein particles (OxLDL). Recently, it was shown that circulating levels of natural anti-PC IgM in LDL receptor-deficient mice increase upon vaccination with S. pneumoniae. Natural IgM antibodies against phosphocholine (anti-PC IgM) resemble C-reactive protein (CRP) regarding specificity and have gained increasing attention because of their supposed role in clearance of damaged cells and in cardiovascular disease. Anti-PC, especially of IgM subclass, is atheroprotective, and predicts a favorable outcome in atherosclerosis development in hypertensives.

**Quality Control**

1. The test results are only valid if the test has been performed following the instructions.
2. All standards and kit controls must be found within the acceptable ranges as stated on the vials.
3. The positive control must be >0.600.
4. Blanks should be <0.500 or lower. Higher blanks or no gradation of A450 values of the calibrators is in indication of high background due to insufficient washing.
5. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

**Species Reactivity**

This kit is tested for human samples and specific for anti-PC IgG.


Alpha Diagnostic Intl. (www.4adi.com) 680-300-PCG/150205A  Page 1
**WORKSHEET OF A TYPICAL ASSAY**

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean A450</th>
<th>Net A450</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Blanks (sample diluent)</td>
<td>0.100</td>
<td>-</td>
</tr>
<tr>
<td>B1, B2</td>
<td>Calibrator A (10 u/ml)</td>
<td>0.396</td>
<td>0.296</td>
</tr>
<tr>
<td>C1, C2</td>
<td>Calibrator B (25 u/ml)</td>
<td>0.598</td>
<td>0.498</td>
</tr>
<tr>
<td>D1, D2</td>
<td>Calibrator C (50 u/ml)</td>
<td>1.37</td>
<td>1.27</td>
</tr>
<tr>
<td>D1, D2</td>
<td>Calibrator D (100 u/ml)</td>
<td>2.77</td>
<td>2.67</td>
</tr>
<tr>
<td>E1, E2</td>
<td>Positive Control</td>
<td>1.75</td>
<td>1.65</td>
</tr>
<tr>
<td>F1, F2</td>
<td>Sample 1</td>
<td>1.55</td>
<td>1.45</td>
</tr>
<tr>
<td>G1, G2</td>
<td>Sample 2</td>
<td>0.56</td>
<td>0.46</td>
</tr>
<tr>
<td>H1, HG2</td>
<td>Sample 3</td>
<td>2.90</td>
<td>2.80</td>
</tr>
</tbody>
</table>

**NOTE:** These data are for demonstration purpose only. It must not be used to determine the sample results.

**CALCULATION OF RESULTS:**

1. Calculate the mean average values of calibrators, controls, and samples.
2. Subtract the blanks values from to obtain net A450.
3. The obtained OD of the standards (y-axis) are plotted against their concentration (x-axis) either on a graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).
4. The concentration of the samples can be read from the standards curve. Results of samples of should be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted to bring the values within the middle of the curve.

**PRINCIPLE OF THE TEST**

The Anti-PC Ig’s ELISA kit is based on the binding of anti-PC Ig’s in samples to PC-antigen immobilized on the microwells; bound anti-PC Ig’s (IgG, or IgM) is detected by antibody-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color (blue) is developed. Stopping Solution is added to terminate the reaction (convert blue to yellow color), and A450nm is then measured using an ELISA reader. Yellow color is directly proportional to the amount of anti-PC present in the sample. The Anti-PC Ig’s concentration in samples is determined relative to supplied anti-PC Ig’s Controls.

**MATERIALS AND EQUIPMENT REQUIRED**

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader.

**PRECAUTIONS**

All human derived material has been tested negative for HIV, HCV, and HBsAg. Nevertheless, all precautions should be taken and samples be treated as potentially hazardous.

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following IS09001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

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
**SPECIMEN COLLECTION AND HANDLING**

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards and controls) have to be diluted with 1X sample diluent (see below).

**Sample Dilution & Antibody Stability**

1. Prepare an initial sample dilution (1:10 or 10 ul sample into 90 ul) of 1X Sample Diluent in order to stabilize antibody activity. Preparation of 1:10 samples enhances reproducible sampling, and stabilizes the antibody activity for months, stored refrigerated until testing complete or store frozen for future testing.

2. Additional sample’s test dilutions of 1:500 should be done in Low NSB Diluent (green) to provide low assay background and good discrimination of specific signal (dilute 1:10 stock 1:50 to prepare 1:500 dilution; example: 5 ul of 1:10 stock in 245 ul Low NSB diluent). It is possible to change the final testing dilution to 1:500-1:2,000 or more depending upon the actual sample background and antibody concentration. All sample dilutions in Low NSB should be at least 5 times the initial dilution and performed the same day as the assay. Do not store test dilutions. Do not store samples diluted in Low NSB diluent beyond the assay date.

**REAGENTS PREPARATION**

1. Prepare 1X Sample Diluent buffer by diluting stock 1:20 with water, (10 ml stock in 190 ml distilled water) Store diluted buffer at 4°C for 1 month. Use 1X sample diluent to prepare sample stock dilutions (1:10) and diluting antibody conjugate.

2. Prepare 1X Wash buffer by diluting stock 1:100 with water, (10 ml stock in 1000 ml distilled water) Store diluted buffer at 4°C for 1 month.

3. Prepare 1X Antibody-HRP Conjugate: Dilute 1:10 with 1X sample diluent (1 ml in 10 ml of 1X sample diluent). Prepare 1 ml for each 8-well strip if using partial strips or 10 ml for a full plate. Prepare only in the required amounts only. Do not store 1x diluted conjugate beyond the assay date.

All reagents must be at room temperature prior to their use.

**STORAGE AND STABILITY**

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or store frozen in small aliquots and should be stable for 3 months.

**TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).**

**Important:** If you have not used this kit before, we recommend to use 1 or 2 strips to run the standards alone to get familiar with the test and not run the risk of making mistakes and lose sample or the whole kit.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. All samples should be diluted 1:500 (5 ul samples in 490 ul sample diluent). It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

1. Dispense 100 ul diluent in 1 well to be used as blank. Pipet 100 ul of ready to use calibrators, controls, and samples (diluted 1:500) into appropriate wells in duplicate. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and incubate at room temp for 60 min.

2. Aspirate the well contents and blot the plate on absorbent paper. Immediately, wash the wells 4 times with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add 100 ul anti-Human IgG-HRP conjugate to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for 30 minutes at room temp (25-28°C).

4. Wash the wells 3 times as in step 3.

5. Add 100 ul TMB substrate solution. Mix gently for 5-10 seconds. Cover the plate and incubate for 20 minutes at room temp. Blue color develops in positive controls and samples.

6. Stop the reaction by adding 100 ul of stop solution to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

7. Measure the absorbance at 450 nm using an ELISA reader within 15 min.

**NOTES**

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Do not touch the bottom of the wells.