OLIGOBIND® APC activity assay
Product no. ADG855
Storage: 2–8°C For Research Use Only!

PRODUCT INSERT ENGLISH

INTENDED USE
The OLIGOBIND® APC activity assay is an enzyme-capture-assay for the quantitative measurement of active Protein C in stabilized plasma samples.

EXPLANATION OF THE TEST
Activated protein C (APC) is a multifunctional serine protease with anticoagulant, anti-inflammatory and anti-apoptotic functions. The active protein is generated from its precursor zymogen protein C (PC) on the surface of endothelial cells by an activation complex formed by thrombin and thrombomodulin (TM). APC released from the endothelial cell surface down-regulates thrombin formation by cleavage of the activated cofactors V and VIII.

A failure to generate sufficient amounts of APC is associated with a prothrombotic and a hyperinflammatory phenotype. The severity of the clinical symptoms depends on the residual APC activity. The prothrombotic phenotype is the leading symptom in milder forms of APC deficiency such as in heterozygous PC deficiency, whereas more severe forms of APC deficiency such as in homozygous PC deficiency are characterized by a thrombo-inflammatory phenotype. Acquired APC dysfunction is critically involved in the pathogenesis of several thrombo-inflammatory diseases including severe sepsis.

In spite of the important physiological functions of APC and its crucial role in the pathogenesis of a variety of diseases, plasma levels of APC have not been considered in diagnostic or therapeutic decision making. In combination with the APC blood collection tubes (Product No. ADG855T) that ensure the stabilization of APC-activity ex vivo, the OLIGOBIND® APC activity assay allows the direct quantification of functional active Protein C in plasma from peripheral blood.

PRINCIPLE OF THE METHOD
Stabilized plasma samples are added to microwells coated with a DNA-aptamer against APC. During an incubation period, APC present in the sample will bind to the aptamer coated to the wells. Following a washing step, a fluorogenic peptide substrate for activated Protein C is added to the microwells. Measuring the change of fluorescence (360 nm/460 nm) nm and extrapolating the value with those of a standard curve determines the level of APC in the plasma sample.

REAGENTS

<table>
<thead>
<tr>
<th>Reagent Name</th>
<th>Description</th>
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<tbody>
<tr>
<td>MTP</td>
<td>Aptamer Coated Microtiter plate, MTP-96 (12x8) well</td>
</tr>
<tr>
<td>WASH</td>
<td>Wash buffer, 50 ml, 1 vial (10x concentrate)</td>
</tr>
<tr>
<td>DILB</td>
<td>Sample dilution buffer, 2 ml, 1 vial (ready-to-use)</td>
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<tr>
<td>CACL</td>
<td>Calcium chloride solution, 0.5 ml, 1 vial (ready-to-use)</td>
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<tr>
<td>STD-50</td>
<td>APC Standard plasma, 50.0 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
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<tr>
<td>STD-25</td>
<td>APC Standard plasma, 25.0 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
</tr>
<tr>
<td>STD-12.5</td>
<td>APC Standard plasma, 12.5 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
</tr>
<tr>
<td>STD-2.5</td>
<td>APC Standard plasma, 2.5 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
</tr>
<tr>
<td>STD-0.5</td>
<td>APC Standard plasma, 0.5 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
</tr>
<tr>
<td>STD-0.1</td>
<td>APC Standard plasma, 0.1 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
</tr>
<tr>
<td>STD-0</td>
<td>APC Standard plasma, 0.0 ng/ml, 0.5 ml, 2 vials (lyophilized)</td>
</tr>
<tr>
<td>ASUB</td>
<td>APC Substrate, fluorogenic substrate, 140 μl, 1 vial (lyophilized), store in the dark!</td>
</tr>
<tr>
<td>SBUF</td>
<td>Substrate buffer, 15 ml, 1 vial (ready-to-use)</td>
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</tbody>
</table>

SPECIMEN COLLECTION AND PREPARATION

Plasma prepared from peripheral blood collected in special APC blood collection tubes (Product No. ADG855T) should be used for this assay. For general sample handling, see "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003.

Plasma collection should be performed as follows:

1. Collect blood into APC blood collection tubes (ref. ADG855T).
2. Drawn blood should be stored cooled (4 °C) and centrifuged (see step 3) within 4 hours.
3. Centrifuge the blood sample at 2,500 x g for 15 minutes.
4. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -20°C for up to 6 months.
5. Frozen plasma should be thawed rapidly at 37°C.

PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

For in vitro use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation.

ASUB - APC Substrate: Reconstitute the APC Substrate with 140 μl deionized/distilled water just prior to use. Let stand for 10 minutes at room temperature (18°-25°C) before gently mixing. DO NOT VORTEX! NOTE: Protect from light. The substrate may be held at room temperature until use. For running all 96 microwells at one time, dilute 100 μL of APC Substrate to 1 mL in Substrate Buffer. If not all 96 microwells are used, dilute 10 μL of APC Substrate to 1 mL in Substrate Buffer for each 8-micro-well strip that will be used. Working strength APC Substrate is stable for 2 hours at 2°-8°C. Discard any unused working strength APC Substrate. Opened APC substrate should be aliquoted and stored in the dark at -20 °C. Avoid multiple freeze-thaw cycles.

MTP Aptamer coated microwells: Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.

WASH Wash buffer: Transfer the content to a 500 mL bottle and fill up the concentrate to 500 mL with filtered deionized/distilled water. Diluted Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.

STD Standards: Reconstitute each APC standard plasma with 50 μl purified, deionised or distilled water, swirl the contents gently and allow the vials to stand at room temperature for at least 15 minutes to ensure complete dissolution. The lyophilised plasma is stable until the date indicated on the vial label when stored at 2°-8°C.

CACL Calcium chloride solution: Supplied ready to use. Opened Calcium chloride solution is stable for 6 month when stored at 2°-8°C.

DILB Sample dilution buffer: Supplied ready to use. Opened Sample dilution buffer is stable for 3 month when stored at 2°-8°C.

SBUF Substrate buffer: Supplied ready to use. Opened Substrate buffer is stable for 3 month when stored at 2°-8°C.
PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided
0.22 µm filtered deionized H₂O
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading fluorescence at 360[ex]/460[em] nm
microwell plate washer

Preparing the APC Standards
1. Reconstitute the APC standard plasmas as instructed under REAGENT PREPARATION AND STORAGE.
   The reconstituted APC standards are ready-to-use. Do not dilute.

Preparation/Recalcification of Samples
2. The plasma samples should initially be tested undiluted. The plasma sample must be recalcified before analysis. Add 2.5 µl of the Calcium Chloride solution to 250 µl plasma sample.

Running standard and samples in duplicate is recommended.

Assay Procedure
3. Open the foil pouch and remove the microwell strips/frame assembly. Remove the strips that will not be used, return them to the foil pouch and tightly reseal the pouch with the desiccant inside. Store the foil pouch at 2 - 8 °C.
4. Pipette 100 µL of the standards or recalcified samples into separate microwells, cover with the acetate sheet and incubate for 1 hour at room temperature (20-25°C) in the dark.
5. Prepare the APC substrate working solution as instructed under REAGENT PREPARATION AND STORAGE. Empty the contents of the microwells with an eight channel multi-pipette using fresh tips for each strip. Subsequently, manually add 250 µl of Wash Buffer to each microwell and wash additional 3 times with Wash Buffer (300 µl / microwell). Remove any remaining droplets by tapping the plate 4-5 times, face down against absorbing material.
6. Place the microwell plate in a fluorescence plate reader set at 360[ex]/460[em] nm and the temperature set at 20-25 °C. Use a low setting for photomultiplier tube (PMT).

Measurement
7. Add 100 µL working strength APC Substrate to each microwell. The reaction begins immediately upon addition of the substrate, measure fluorescence at room temperature at 360[ex]/460[em] nm within 1-2 minutes (T=0).
8. Measure the increase in fluorescence over 120-240 minutes, collecting data at 30 minutes intervals. Take the linear part of the curve and calculate the rate of change in fluorescence (dFU/min).

RESULTS

A standard curve is constructed by plotting the mean change of fluorescence (dFU) for each standard versus the corresponding concentration of APC in ng/mL. A standard curve should be generated each time the assay is performed. A representative standard curve using a Fluostar Optima Fluorometer (BMG Labtech) is shown below and is for demonstration purposes only.

Representative Standard Curve

VALUES

BIBLIOGRAPHY

2. The Protein C pathway. Esmon CT, Chest 2003; 124: 26S–32S.