Human Protein C ELISA Kit

Enzyme Immunoassay for the quantitative determination of Human Protein C in citrated plasma.

Catalog number: ARG81037

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Protein C is a vitamin K-dependent serine protease that regulates blood coagulation by inactivating factors Va and VIIIa in the presence of calcium ions and phospholipids (PubMed:25618265). Exerts a protective effect on the endothelial cell barrier function (PubMed:25651845). [provide by uniprot]

PROC gene encodes a vitamin K-dependent plasma glycoprotein, protein C. Protein C is cleaved to its activated form by the thrombin-thrombomodulin complex. This activated form contains a serine protease domain and functions in degradation of the activated forms of coagulation factors V and VIII. Mutations in this gene have been associated with thrombophilia due to protein C deficiency, neonatal purpura fulminans, and recurrent venous thrombosis. [provided by RefSeq, Dec 2009]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Protein C has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Protein C present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for Protein C is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Protein C bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Protein C in the sample is then determined by comparing the O.D. of samples to the

standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Standards	3 X 0.4 ml (lyophilized)	4°C
Control N	3 X 0.4 ml (lyophilized)	4°C
Control D	3 X 0.4 ml (lyophilized)	4°C
HRP-Protein C antibody Conjugate	15 ml (ready to use)	4°C
5X Sample buffer	20 ml	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- All kit reagents and specimens should be brought to room temperature (20-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens. For automated systems, we recommended incubated the plate at 23°C.
- Never expose components to higher temperature than 37°C.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.

- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Store the unopened reagents at 2 - 8°C until expiration date. Once opened the reagents are stable for 2 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using sodium citrate (3.2% or 3.8%) as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at 4°C for up to 8 hours or store at ≤ -20°C for longer periods. Avoid repeated freeze-thaw cycles.

Note:

Do not use haemolytic, icteric or lipaemic specimens.

Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **Sample buffer:** Dilute 5X Sample buffer into distilled water to yield 1X Sample buffer. (e.g. 20 ml of 5X Sample buffer + 80 ml of distilled water) The diluted 1X Sample buffer are stable for 1 month at 2-8°C.
- **Wash buffer:** Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. 20 ml of 50X Wash buffer + 980 ml of distilled water) The diluted 1X Wash buffer are stable for 1 month at 2-8°C.
- **Reference Plasma:** : Reconstitute the lyophilized contents of the Reference Plasma with 0.4 ml distilled water and let the Reference Plasma stand in the vials for 10 minutes at room temperature. Mix several times before use. The reconstituted standards are stable for 8 hours at 2-8°C.

  Before serial dilutions of reference plasma, the reference plasma should be diluted at a 1:2 dilution with 1X sample buffer and mix well, e.g. 100 µl reference plasma + 100 µl sample buffer.

The dilution set is prepared by using the prediluted Reference Plasma.

Volume of Reference Plasma (1:2 pre-diluted) (µl)	Volume of Sample Buffer (1X) (µl)	Reference Level
60	1000	150%
40	1000	100%
30	1000	75%
20	1000	50%
10	1000	25%
10	2000	12.5%

- **Controls:** Reconstitute the lyophilized contents of the Control N (Normal plasma) and Control D (deficient plasma) with 0.2 ml distilled water each and let the controls stand in the vials for 10 minutes at room temperature. Mix several times before use. The reconstituted Controls are stable for 8 hours at 2-8°C. Before assay, the controls should be pre-diluted at 1:51 dilution with 1X sample buffer and mix thoroughly. (e.g. 20 µl of control + 1000 µl of 1X sample buffer)
- **Sample:** Before assay, the samples should be pre-diluted at 1:51 dilution with 1X sample buffer and mix thoroughly. (e.g. 20 µl of plasma sample + 1000 µl of 1X sample buffer) If the initial assay found samples contain Protein C higher than the highest standard, the samples can be diluted with Standard 0 and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly).

  b) Dilution 1:100: 10 µL 1:10 diluted a) + 90 µL Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-26°C) before use. Reference Plasma, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **prediluted samples** in duplicate into the appropriate wells.
3. Add **100 µl** of **Reference Plasma and controls** in duplicate into the appropriate wells.
4. Gently tap the plate to mix well. Incubate for 30 min at RT (20-26°C).
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X wash buffer** (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 20 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of **HRP-Protein C antibody** into each well. Incubate for **30 minutes** at **RT.**
7. Aspirate each well and **wash as step 5**
8. Add **100 µl** of **TMB substrate** to each well. Incubate for **30 minutes** at **room temperature** in dark.
9. Add **100 µl** of **Stop Solution** to each well, using the same order as pipetting the substrate. Incubate for 5 minutes at room temperature in dark. Gently tap the plate to mix well.
10. Read the OD with a microplate reader at **450 nm** immediately. (Optional:

read absorbance at 620 nm as reference wavelength) It is recommended that the wells should be read within 30 min after adding Stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log linear graph paper (log/lin), construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. From the O.D. of each sample, read the corresponding patient relative value expressed in %. Multiply the patient relative value obtained from the reference curve by the assigned factor referred in the quality control leaflet to calculate the Protein C antigen level in % of normal.

6. Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

7. For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

8. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

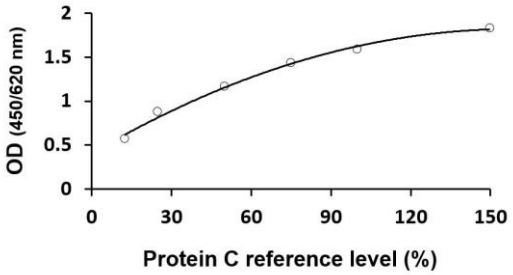
9. In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

10. The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

11. If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Expected values

The values for Protein C are given in relative percent (%) as compared to pooled normal plasma. The Protein C concentration in normal human plasma ranges usually between 70 % and 140 %. Samples with values above the range of the reference curve may be assayed again at higher dilutions for accurate results. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

Sensitivity

Testing sample buffer 30 times on Protein C gave an analytical sensitivity of 6.0%.

Assay Range

12.56-150%

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 3.03% and the CV value of inter-assay precision was 4.5%.

Linearity

94.6-105.7%