TECHNOZYM® Protein C ELISA

For research use only

GB

REF TC12021 TECHNOZYM® Protein C ELISA

Symbols key			
	Manufacturer		Expiry date
	Storage temperature		Consult instructions for use
AQUA	Distilled water		Determinations
BUF	Reaction buffer	LOT	Lot
CAL	Calibrator	MTP	Microtiter plate
CONJ	Conjugate	REF	Catalogue number
CONT	Control	RTU	Ready to use
DIL	Dilute or dissolve in	STOP	Stop solution
INC	Incubation buffer	SUB	Substrate
RUO	For research use only	WASH	Washing solution concentrate

Technoclone Herstellung von
Diagnostika und Arzneimitteln GmbH
Brunner Str. 67 - 1230 Vienna, Austria

TECHNOZYM® Protein C ELISA

GB

PRODUCT DESCRIPTION

INTENDED USE

The TECHNOZYM® Protein C ELISA is a chromogenic test for the determination of Protein C concentration in human plasma.
Protein C is a vitamin K dependent serine protease which, when activated, inhibits coagulation by inactivating the clotting factors V/Va and VIII/VIIIa. Additionally, protein C has been shown to have profibrinolytic activity. Hereditary, heterozygous protein C deficiency has been found to be associated with an increased risk of venous thrombosis and hereditary, homozygous total protein C deficiency has been found in neonates with purpura fulminans. Reduced levels of protein C have been found in association with vitamin K deficiency and during coumarin therapy.

COMPOSITION

1. ELISA test strips (12), with 8 wells each, coated with a monoclonal anti-Protein C antibody; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 vial, 80 mL.
3. Incubation buffer (= sample dilution buffer) (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin; and dye, 1 vial, 90 mL, ready for use.
4. Calibrators (Standards) numbered from 1 to 5; lyophilised; 1 vial each; 0.5 mL. **Concentrations are lot-specific; consult the label on the vial.**
5. High and Low control plasma; lyophilised; 1 vial each; 0.5 mL. **Concentrations are lot-specific; consult the label on the vial.**
6. Conjugate: polyclonal anti-Protein C POX; dyed blue; 1 vial, 0.3 mL.
7. Chromogenic substrate TMB (tetramethylbenzidine); 1 vial; 12 mL; ready to use.
8. Stopping solution sulphuric acid 0,45 mol/L; 1 vial; 12 mL; ready to use.
9. Adhesive film: for ELISA test strips; 2 pieces.

MATERIAL REQUIRED (not supplied with the kit)

1. Distilled water
2. Test tubes for diluting standards and samples
3. Measuring cylinder (1000 mL)
4. Precision pipettes (50, 100 and 1000 µL)
5. Variable pipette (100 and 1000 µL)
6. Multichannel and/or dispensing pipettes (100 and 200 µL)
7. ELISA washer or multichannel pipette
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available.

WARNING AND PRECAUTIONS

- For research use only
- All human blood or plasma products as well as samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas are made from human blood and any individual plasma involved in the procedure is HBsAg, HIV 1/2 Ab and HCV-Ab-negative (see labels on the vials). However, all human blood products should be handled as potentially infectious material.
- Stopping solution may irritate the skin. Should acid get into your eyes, wash out immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes!

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened vial at + 2…8 °C.
Stability after reconstitution/opening:

Material/Reagent	State	Storage	Stability
Calibrators, control plasmas	after reconstitution	-20°C	6 months
ELISA test strip	after opening	+2...8 °C with adhesive film in plastic bag with drying agent	expiry date
Washing buffer conc.	after opening	+2...8°C	6 months
Washing buffer	1+11.5 dilution of concentrate	+2...8 °C	3 weeks
Incubation buffer (= sample dilution buffer)	after opening	+2...8 °C	2 months
Conjugate	after opening	+2...8 °C	6 months
	working solution	room temperature +18…25°C	60 minutes
Chromogenic substrate TMB	after opening	+2...8 °C	expiry date

TEST PROCEDURE

PREPARATION OF THE SAMPLES

Sample material: Human citrated plasma. Samples may be stored for three hours at room temperature. At -20°C they can be stored for one month. Samples may not be frozen and thawed several times.

PREPARATION OF REAGENT

1. Before starting the test, all the required components are to be brought to room temperature.
2. Preparing the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well! (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37°C within 10 minutes.
3. Reconstituting calibrators and control plasmas: Calibrators and control plasmas are reconstituted with **500 µL** distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
4. **Calibrator dilution (1+30):**
   Dilute 10µL Calibrator with 300µL incubation buffer. Mix for 10 seconds
5. **Control plasma and sample dilution (1+50)**
   Dilute 10µL of Control plasma/sample with 500µL incubation buffer. Mix for 10 seconds.
6. Preparing the **conjugate working solution (1+50):** Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

For 8 test wells: Mix 20 µL conjugate with 1000 µL incubation (= sample dilution) buffer

PERFORMANCE OF THE TEST

SAMPLE INCUBATION (reference 1,2,6)	Pipette **diluted calibrators, control plasmas**, and **samples** into test wells; cover test strips with film	**100 µL**
	Incubate at **room temperature**	**60 minutes**
WASHING (reference 1,3,4)	**Washing buffer**	**3 x 200 µL**
CONJUGATE REACTION (reference 1,2)	Pipette **conjugate working solution** into wells, cover test strip with film	**100 µL**
	Incubate at **room temperature**	**60 minutes**
WASHING (reference 1,3,4)	**Washing buffer**	**3 x 200 µL**
SUBSTRATE REACTION (reference 1,2)	Pipette **Substrate solution** into test wells, cover test strip with film	**100 µL**
	Incubate at **room temperature**	**30 minutes**
STOPPING (reference 1,2)	Pipette **stopping solution** into wells	**100 µL**
MEASURING (reference 5)	ELISA-Reader, **450 nm**	shake 10 sec., measure within 10 min.

References

1. Reagents of different lots must not be combined
2. Precision and performance, among others, essentially depend on the following factors:
   - Thorough mixing of all substances used for dilution, 10 sec. with Vortex Mixer
   - Test calibrators, controls and samples in duplicates
   - Incubate at indicated temperature (RT: room temperature: +18…25°C)
   - Strict observance of the order of pipetting and of the time element as indicated
   - The time for sample incubation, conjugate and substrate reaction as indicated starts after pipetting the last sample. Incubation times should not vary by more than ± 5%.
   - During sample incubation and conjugate reaction, the time for pipetting calibrators/control plasmas/samples and/or conjugate solutions must not exceed 60 seconds per ELISA test strip (8 wells).
   - During substrate reaction and at stopping, the time needed for pipetting the substrate and/or the stopping solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel- and dispensing pipettes.
3. Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
4. After the last washing, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper; by gentle tapping, the last remnants must be removed.
5. By measuring the difference in wave lengths at 450 and 620 nm the precision of the test is increased.
6. A calibration curve has to be created for every assay

LIMITATION OF THE TEST

As with any assay employing antibodies from an animal source to capture a target molecule, the possibility exists for interference with the serum or plasma of samples who have been exposed to preparations containing animal antibodies. Falsely elevated or depressed values may be seen in these samples.

ANALYSES RESULTS

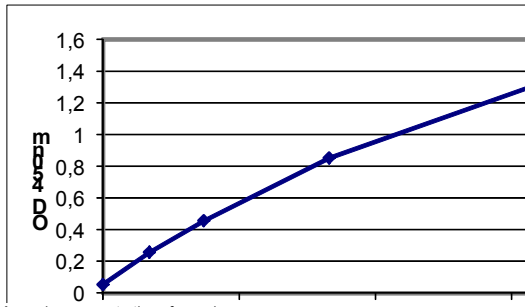
CALCULATION OF THE RESULTS

Setting up a reference curve: X axis: concentration Protein C Antigen (IU/mL)
Y axis: Extinction at 450nm

Graph plot is linear-linear with a point to point or cubic spline fit.

Assessment of reference curve
The validity of the test may be checked on the basis of the calculated control values.

Example of standard curve



Measuring concentration of samples

- Read off the concentration from the reference curve.
- Multiply concentration by 1.7 (as calibrator and sample dilution differ by this factor)
- If there are samples with extinction coefficients higher than that of the highest point on the curve, they have to be prediluted with incubation buffer (1+1, or 1+3). The measured concentration then has to be multiplied with the dilution factor 2 or 4, respectively.

INTERPRETATION OF RESULTS / REFERENCE RANGE

Normal range for Protein C concentration: 0.70 – 1.60 IU/mL (n=54).
It is recommended that individual laboratories establish their own normal range. When interpreting the results the history of the sample has to be taken into account.

STANDARDISATION

The calibration material used is the WHO International standard Protein C in plasma (human).
1 IU/mL equates to 100 % Protein C antigen.

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

PRECISION

Reproducibility was determined with different samples (in series and day to day).
The following results were obtained:

	Intra assay		Inter assay	
Sample	Sample 1	Sample 2	Sample 3	Sample 4
n	96	66	23	23
MV (IU/mL)	0.91	1.02	0.74	0.18
SD (IU/mL)	0.057	0.060	0.048	0.012
CV (%)	6.31	5.81	6.4	6.5

COMPARISON OF METHODS OR CORRELATION

Following correlation (%) was obtained in comparing TECHNOCHROM® Protein C (Technoclone) on a Trinity AMAX coagulation analyzer with the TECHNOZYM® Protein C ELISA:

n=55 y = 0.90x + 13.37 R = 0.859

ASSAY RANGE

0.0165 – 1.90 IU/mL
However, the effective range of reach test run will depend on the assayed value of calibrator 1.

DETECTION LIMIT

0.0165 IU/mL

LITERATURE

1. *Clouse, LH, Comp, PC. The regulation of hemostasis:The Protein C System. N Engl J Med 314:1298-1304,1986.*
2. *Mannucci PM, Vigano S. Deficiencies of protein C, an inhibitor of blood coagulation. Lancet. 1982 Aug 28;2(8296):463-7.*
3. *Stenflo, J. Structure and function of Protein C. Semin, Thromb Haemost 10:109-121, 1984.*
4. *Griffin, JH, Evatt, B, Zimmerman, TS, et al. Deficiency of Protein C in congenital thrombotic disease. J Clin Invest 68:1370-1373, 1981.*
5. *Tollefson, DFJ, Friedman, KD, et al. Protein C Deficiency: A cause of unusual or unexplained thrombosis. Arch Surg 123:881- 884, 1988.*
6. *Preissner, KT. Biological relevance of the Protein C system and laboratory diagnosis of Protein C and S deficiencies. Clin Science 78:351-364, 1990*