TECHNOCHROM® C1-INH
For research use only

technoclone
TC

GB

REF | 5345003 TECHNOCHROM® C1-INH $\overline{\underset{30}{\Sigma}}$

	Symbols key		
▲ (Manufacturer symbol)	Manufacturer	⊠	Expiry date
(thermometer symbol)	Storage temperature	📖	Consult instructions for use
AQUA	Distilled water	$\underset{\Sigma}{\nabla}$	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

▲ (Manufacturer symbol) Technoclone Herstellung von
Diagnostika und Arzneimitteln GmbH
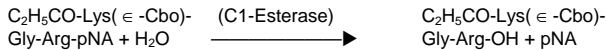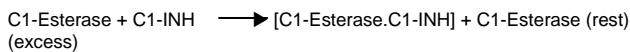Brunner Str. 67 - 1230 Vienna, Austria

TECHNOCHROM® C1-INH

PRODUCT DESCRIPTION

INTENDEND USE

For determination of C1-INH in a sample.
The C1-esterase inhibitor (C1-INH) is a regulatory protein that functions as an inhibitor of several serine proteases in the complement system, the kallikrein-kinin system, the coagulation cascade and in fibrinolysis.

TEST PRINCIPLE

C1-Esterase + C1-INH $\longrightarrow$ [C1-Esterase.C1-INH] + C1-Esterase (rest)
(excess)

C_2H_5CO-Lys($\in$ -Cbo)-   (C1-Esterase) C_2H_5CO-Lys($\in$ -Cbo)-
Gly-Arg-pNA + H_2O $\longrightarrow$ Gly-Arg-OH + pNA

COMPOSITION

Reagent kit for 30 photometric C1-Esterase Inhibitor determinations.

mL	reagent	other data
1 x 3	Substrate C1-1	18 µmoL, AcOH. C_2H_5CO-lys ($\in$ -Cbo)-Gly-Arg-pNA, p.m. = 729.8
1 x 3	C1-Esterase	human
1 vial	Coagulation Reference for C1-INH	125% = 1.25 IU/mL C1-INH (See label for reconstitution volume)
1 x 1	Coagulation Control A for C1-INH	lyophilized abnormal plasma
1 x 1	Coagulation Control N for C1-INH	lyophilized normal plasma
1 x 25	Sample Buffer A	Tris (6.1 g/L)-NaCl (15 g/l)-Buffer pH 7.4
1 x 20	Reaction Buffer B	Tris (6.1 g/L)-NaCl (15 g/l)-Buffer pH 8.5

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes
- Distilled water

WARNING AND PRECAUTIONS

- For research use only
- All blood and plasma samples and products have to be regarded as potentially infectious and handled with appropriate care and in compliance with the biosafety regulations in force and must be disposed of in the same way as hospital waste.
- Each single donor plasma and each lot of Coagulation Control are tested and found negative for Hb_sAg, HIV 1/2 Ab and HCV Ab. However, universal precautions (treating all human source materials as if potentially infectious) should be exercised.

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at +2…8 °C.

Stability after reconstitution:
The reconstituted reagents are stable for 6 hours at reaction temperature.
Reconstituted reagents may be stored at –20°C.
The vials can be only frozen once. Upon storage, caps should be screwed tightly.
Frozen reagents should be used within one month.
DO NOT FREEZE THE SUBSTRATE -BUFFER MIXTURE

TEST PROCEDURE

PREPARATION OF PLASMA SAMPLES

Plasma separation:
Mix 9 parts of venous blood and 1 part sodium citrate solution (0.11 mol/L) and centrifuge for 15 minutes at a RCF of at least 2500 (corresponding to DIN 58905). The plasma sample may not be stored at room temperature for more than three hours; otherwise the sample has to be frozen immediately after centrifugation.

Sample preparation:
Before testing the plasma samples are diluted with Sample Buffer A at a ratio of 1:11 (0.05 mL sample + 0.50 mL Buffer A). Samples with C1-INH activity > 125%-should be diluted 1:22.

PREPARATION OF REAGENT

All reagents including distilled water should have reached room temperature before use. The lyophilized reagents are dissolved in the volume of distilled water indicated and are ready for use after 10 minutes. For standardization test a reconstitution time of 30 min is recommended.

PERFORMANCE OF THE TEST

C1-Esterase and the diluted sample are kept at room temperature, the Substrate-Buffer mixture at +37°C. Measurements are done at +37°C.

Mixing the Substrate C1-1 with reaction Buffer B:

Kinetic determination	
1 part	Substrate C1-1
5 parts	Reaction Buffer B

Pipetting scheme: Pipette into plastic tubes or cuvettes.

Kinetic Determination		Kinetic Determination in microplate
100 µL	diluted sample	50 µL
100 µL	C1-Esterase	50 µL
5 minutes	incubation +37°C	5 minutes
600 µL	Substrate-buffer mixture	300 µL

The extinction increase is measured at 405 nm at +37°C. During 4 min the reaction is linear.

LIMITATION OF THE TEST

In inflammatory processes the activity of the acute-stage-protein C1-INH may be far above the normal value. It is recommended to test samples with values above 125% C1-INH once more in a dilution of 1:22.

ANALYSES RESULTS

CALCULATION OF THE RESULTS

To establish a reference curve 3 serial dilutions of Coagulation Reference are prepared and tested together with an optional blank (sample buffer A) reading.

Predilute the Coagulation Reference 1:11 with sample buffer A (0.05 mL plasma + 0.50 mL buffer A). From this predilution prepare a series of dilutions (1:1, 1:2, 1:4) also with sample buffer A (the 1:1 dilution corresponds to the 1:11 predilution). This series should be tested in the same way as a sample in the assay. The absorption increase ($\triangle$A/min) is plotted on linear graph paper as readings for 125, 62.5 and 31.25 % C1-INH value and are plotted to give a linear calibration curve. The blank reading may be used in the reference curve as the 0 %-C1-INH value.

The control plasmas Coagulation Control A and N are prediluted 1:11 (0.05 mL plasma + 0.50 mL Buffer A) with sample buffer A and may be read off directly from the reference curve. The values should fall within the confidence limits printed on the label of the control plasma.

Example: manual method:
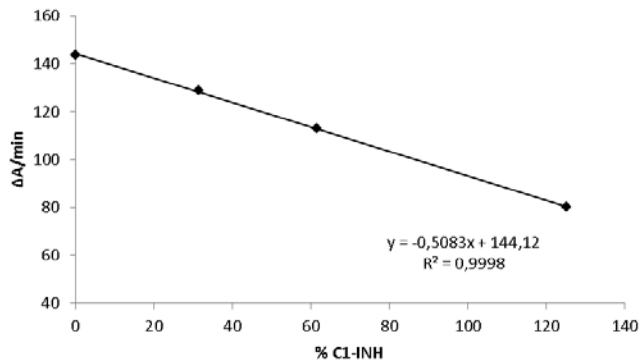
All samples diluted 1:11 can be direcly read off from the calibration curve.
For samples diluted other than 1:11 the %–activity read off from the calibration curve has to be converted as follows:

$$\frac{\% \text{ C1-INH (calibration curve)}}{11} \times \text{actual dilution ratio} = \% \text{ C1-INH of sample}$$

Thus in samples diluted 1:22 the C1-INH activity is twice the value read off from the calibration curve.

REFERENCE RANGE

70 – 130 % of normal C1-INH (0.70-1.30 IU /mL)

STANDARDIZATION

The Reference Standard C1 INH is calibrated against WHO plasma standard.
Concentrations are lot-dependent, consult the label on the vials.

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 1	sample 2
n	12	12	6	6
Mean %	98	53	96	54
SD (%)	3.96	2.61	2.49	2.29
CV (%)	4.02	4.89	2.58	4.2

COMPARISON OF METHODS OR CORRELATION

Following correlation (%) was obtained in comparing TECHNOCHROM C1INH with:

C1INH EIA (Quidel) $\qquad$ $y = 0.8287x +11.493$ $R^2 = 0.81$

LITERATURE

Please contact Technoclone or your local distributor for literature or technical applications for the test.