TECHNOCHROM® F VIII:C f. Ceveron®

For research use only

GB

DE

REF 5344103 TECHNOCHROM® F VIII:C f. Ceveron®

symbols key / Symbolschlüssel			
	Manufacturer	AQUA	distilled water
	expiry date	DIL	dilute or disolve in
	storage temperature	LOT	lot
	consult instructions for use	REF	catalogue number
RUO	for research use only		
	determinations		

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

TECHNOCHROM® F VIII:C f. Ceveron®

GB

PRODUCT DESCRIPTION

INTENDEND USE

TECHNOCHROM® F VIII:C f. Ceveron® contains reagents for the photometric determination of Factor VIII activity in plasma and for plasma derivatives. It can be used for assaying Factor VIII deficiencies as well as monitoring Factor VIII substitution therapies.

TEST PRINCIPLE

$$FVIII_{sample} + (FIX_{a\beta}\text{-}PL\text{-}Ca^{++}) \xrightarrow{F\ IIa} FVIII_a\text{-}FIX_{a\beta}\text{-}PL\text{-}Ca^{++}\text{-complex}$$

$$F\ X \xrightarrow{F\ VIII_a\text{-}F\ IX_{a\beta}\text{-}PL\text{-}Ca^{++}\text{-complex}} FXa$$

$$Substrate\text{-}FX_a\text{-}1 \longrightarrow Substrate\text{-}FX_a\text{-}1 + pNA$$

COMPOSITION

REF 5344103: Reagent kit for 2x40 photometric FVIII:C determinations

mL	reagent	other data
2 x 2	Substrate FXa-1+ αNAPAP	10 µmoL / vial F Xa-1, 0.012 µmoL / vial αNAPAP
2 x 2	Reagent A	Phospholipid, albumin
2 x 2	Reagent B	F $IX_{aß}$, F X, Ca^{++}, albumin, thrombin
1 x 1	Ref. Stand. FVIII 1	~ 130 % or 1.30 IU FVIII / mL
1 x 1	Ref. Stand. FVIII 2	~ 70 % or 0.70 IU FVIII / mL
1 x 1	Ref. Stand. FVIII 3	~ 10 % or 0.1 IU FVIII / mL
1 x 1	Ref. Stand. FVIII 4	≤ 0.5 % or 0.005 IU FVIII / mL
2 x 20	F VIII dilution buffer	3.4 g/L Imidazole, 5.85 g/L NaCl, 0.2% Albumin, pH 7.4
2 x 8	F VIII reaction buffer	6.06 g/L Tris, 3.03 g/L Na_2ETDA, 25 g/L NaCl, pH 8.3

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes
- Distilled water
- Control Plasma Normal and Abnormal

REF	5020020	Coagulation Control N f. Ceveron	5 x 1 mL
REF	5020025	Coagulation Control N f. Ceveron	50 x 1 mL
REF	5021035	Coagulation Control A f. Ceveron	5 x 1 mL
REF	5021040	Coagulation Control A f. Ceveron	50 x 1 mL

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 Reference Standards 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:

Stability	+20°C	Ceveron® (12° C)	+4°C	-20°C
substrate	1 month	1 month	6 months	1 year
Reagent A	17 hours	24 hours	2 days	14 days
Reagent B	8 hours	24 hours	2 days	14 days
Ref. Standards F VIII	8 hours	8 hours	2 days	1 month
substrate-buffer-mixture	8 hours	-	-	-

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 g (corresponding to DIN 58905). The plasma sample may not be stored at room temperature for more than 3 hours; otherwise the sample has to be frozen immediately after centrifugation. Stability at –20°C is 1 month.
Samples, standards and controls are used undiluted, dilution is made by the instrument.

PREPARATION OF REAGENT

The lyophilized reagents are dissolved in the volume of distilled water indicated on the vial and are ready for use after 10 minutes. For standardization tests a reconstitution time of 30 min is recommended.
Reconstitute the Substrate for Factor Xa with 2 mL distilled water. The reconstituted substrate (2ml) is diluted with 8 mL FVIII Reactionbuffer (1+4)
Reconstitute the Reagent A with 2 mL distilled water. Reconstitute the Reagent B with 2 mLdistilled water. FVIII Dilution Buffer buffer and FVIII Reaction buffer are ready to use
Reconstitute the Reference Standard FVIII 1-4 with 1.0 mL distilled water. For quality control reconstitute the Coagulation Control N and the Coagulation A with 1.0 mL distilled water.

PERFORMANCE OF THE TEST

Technoclone provides application sheets for Ceveron®. The application sheets contain analyser/assay specific handling and performance information. Please consult also the instruction manual of the Ceveron®.

Pipetting scheme for Ceveron alpha:

Kinetic determination	
50 µL 50 µL 50 µL	diluted sample Reagent A Reagent B
300 seconds 250 µL	incubation 37°C Substrate-buffer-mixture

LIMITATION OF THE TEST

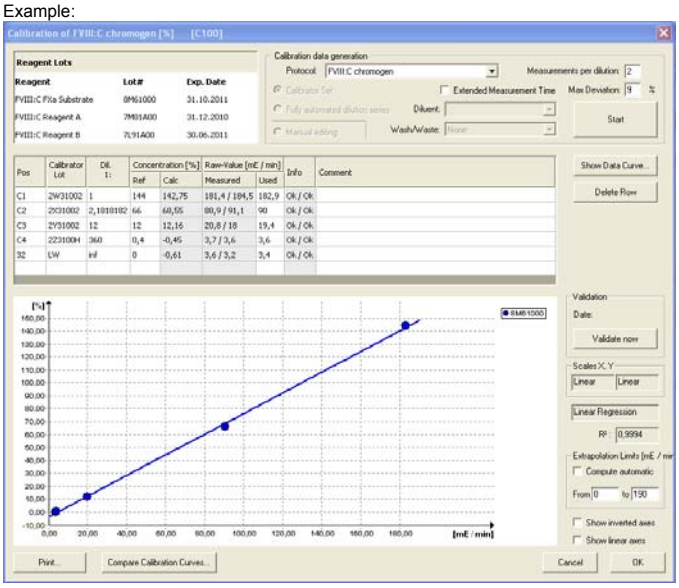
- The values found when testing Coagulation Control N and Coagulation Control A should be compared to the value given on the data-key for the corresponding lot of Control plasma.
- If the results obtained are outside the recommended range, avoid measuring samples until the problem is solved.
- A new calibration is required for each batch of TECHNOCHROM® F VIII:C and for each instrument used. Also a new calibration is recommended, if software changes are introduced or following a major service of either instruments or equipment.

ANALYSES RESULTS

CALCULATION OF THE RESULTS

With the FVIII Reference Standards a 5 point calibration curve is made. The last point is a preparation of the dilution buffer.To establish a calibration curve please follow the instruction in the Ceveron® application sheet for TECHNOCHROM® F VIII:C calibration.

Example:



NORMAL RANGE

0.6 – 1.5 IU F VIII (60 - 150% of normal)[1]

STANDARDIZATION

The Reference Standards F VIII were calibrated against the 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	11	12	10	12
MV (%)	81.8	22.4	102.8	29.8
SD (%)	4393	1.1218	4.857	1.319
CV (%)	5.37%	5.01%	4.72%	4.42%

COMPARISON OF METHODS OR CORRELATION

Following correlation (%) was obtained in comparing TECHNOCHROM® FVIII:C (Technoclone) with the FVIII deficient Plasma Imidazole buffer method:

n=42 y = 1.0102x – 0.8464 R^2 = 0.9568

LINEARITY

0.0 – 144 (Activity %)

DETECTION LIMIT

0% (Activity %)

LITERATURE

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

(1) H.Lang, M. Oberreiter, B. Moritz: A new Chromogenic Factor VIII:C-Assay Based on a System of Human Proteins. Thromb. Haemostas. Abstracts XIII[th] Congress ISTH, Th 65 (6), 943 (1991).