Factor VIII Inhibitor Reagent Kit
For research use only

GB

REF 5152005 Factor VIII Inhibitor Reagent Kit (Bethesda Units) Σ 2-4

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

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Factor VIII Inhibitor Reagent Kit (Bethesda Units)

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PRODUCT DESCRIPTION

INTENDEND USE

Reagent kit for carrying out Factor VIII inhibitor assays.

COMPOSITION

The Factor VIII Inhibitor Reagent kit for 2-4 determinations contains:

vial(s)	Reagents
2 x	Factor VIII Normal Plasma ~ 3 mL
1 x	Factor VIII Inhibitor Plasma 1 mL
1 x	Factor VIII Inhibitor Free Plasma i.ads. 1 mL
1 x	Imidazole buffer, sterile 17 mL

The reagents for Factor VIII assay are not included.

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes - Distilled water
- Solutions/buffers:

REF	5277015	$CaCl_2$ 25 mmol/L Solution	100 mL
REF	5410010	Imidazole Buffer	50 mL

- Reagents

REF	5154007	F VIII Deficient Plasma, native	5 x 1 mL
REF	5035060	Dapttin TC	5 x 2 mL
REF	5035090	Dapttin TC	6 x 10 mL

- Chromogenic method

REF	5344101	Technochrom F VIII:C	40 tests
REF	5344103	Ceveron Technochrom F VIII:C	40 tests

- Calibration Plasma

REF	5220110	Coagulation Reference	5 x 1 mL
REF	5220130	Ceveron Coagulation Reference	5 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.
- This lot of reagents prepared from human blood and each single plasma used for this lot are HBsAg, HIV 1/2 Ab negative and HCV Ab positive. At present plasma of haemophiliacs is only available as HCV Ab positive (see package label and vial label).

STABILITY AND STORAGE

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

Stability after reconstitution: *RT= room temperature

RT*	-20 °C
2 hours	1 month

TEST PROCEDURE

PREPARATION OF PLASMA SAMPLES

Plasma separation:
Mix 9 parts of venous blood and 1 part of Sodium Citrate Solution (0.11 mol/L) and centrifuge for 15 min at a RCF of at least 2500 (corresponding to DIN 58905). The plasma sample can be stored at room temperature for 3 hours. For longer storage freeze immediately after centrifugation (1 month).

PREPARATION OF REAGENT

Factor VIII Normal Plasma, 2 vials, to be dissolved with the amount of distilled water indicated on the label. The normal plasma then contains one I.U. Factor VIII/mL.
Factor VIII Inhibitor Plasma, to be dissolved in 1 mL distilled water. The number of Factor VIII inhibitor units/mL (Bethesda units) is given on the label.
Factor VIII Inhibitor Free Plasma i.ads., to be dissolved in 1 mL distilled water. It serves as a negative control without Factor VIII Inhibitor.

DETERMINATION OF FACTOR VIII

The Factor VIII determination has to be effected either by the one-stage method or by using a chromogenic method (TECHNOCHROM F VIII:C).
When setting up the calibration curve with the one-stage method, Coagulation Reference shall be applied.

DESCRIPTION AND CALCULATION OF THE TEST

CEVERON

Technoclone provides Application sheets for Ceveron® alpha. The Application sheets contain analyser/assay specific handling and performance information which may differ from that provided in this instruction for use. In this case the information contained in the Application sheets supersedes the information in this instruction for use. Please consult the instruction manual of the Ceveron® alpha.

MANUAL

1. **Test sample:** The citrated plasma, either neat or diluted with Imidazole buffer is mixed in equal parts with the Factor VIII Normal Plasma (1 I.U. Factor VIII/mL).
2. **Normal value (comparison mixture):** The normal plasma is diluted with equal parts of Imidazole buffer in a similar way to the test sample.
3. **Test:** Each mixture has to be incubated for exactly two hours in a water bath at 37°C and stored at **2…8°C (ice waterbath)** until testing of the F VIII content. This test than has to be performed within two hours. The incubated sample to be tested is diluted in accordance with the selected F VIII method (one stage method: 1:5 with imidazole buffer dilution, TECHNOCHROM F VIII:C 1:41 with F VIII dilution buffer). In order to set up the F VIII calibration curve, TECHNOCLONE's F VIII standard plasmas calibrated against the valid WHO plasma standards should be used (not contained in the kit).
4. **Residual % Factor VIII Activity:** A test sample has no inhibitor if the Factor VIII value of the test sample is the same as the value of the control mixture i.e. the % Factor VIII activity remaining after incubation is 100%.

$$\%\ \text{F VIII residual activity} = \frac{\text{F VIII value of the test sample}}{\text{F VIII value of the comparison mixture}} \times 100$$

5. **Definition of Bethesda Unit:** A test sample treated by the method above with a residual Factor VIII activity of 50% contains one Bethesda Unit of Factor VIII Inhibitor/mL.
6. **Calculation:** Using semi log graph paper, residual % Factor VIII activity is plotted against Bethesda Units/mL (Linear Axis). The residual % Factor VIII activity between 25% and 75% can then be read off and the number of units Factor VIII Inhibitor/mL is multiplied by the dilution factor to obtain the number of Bethesda units per mL in the sample (see graph.). For this purpose it is also possible to make use of the equation below which is based on the graphic presentation:

$$\text{F VIII Inhibitor (BU)} = \frac{[2\text{-log (residual activity F VIII)}]}{0.30103}$$

INTERPRETATION OF RESULTS

A. Samples: The plasma samples under test can be classified in three groups (a) without Factor VIII inhibitor, (b) weak Factor VIII inhibitor (≤ 10 BU./mL) and (c) strong Factor VIII inhibitor. There are different dilutions for each test group (see table).
In special cases other dilutions may be used e.g. 1:1.5 or 1:512 and 1:1024.

	B.U./mL	undil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
(a) without F VIII-Inh.	≤ 1	+	+							
(b) weak F VIII-Inh.	≤ 10	+	+	+	+	+				
(c) strong F VIII-Inh.	> 10				+	+	+	+	+	+

Geometric dilution of the plasma sample under test, with an unknown value of Factor VIII inhibitor, are made with imidazole buffer based on the group class either a, b or c. Then one part of sample (0,2 mL) is mixed with one part of Factor VIII Normal plasma (0.2 mL).

B. Factor VIII Inhibitor Plasma: Three geometrical dilutions of the Factor VIII Inhibitor Plasma with known Bethesda Units should be used as a positive Control. The dilution factor of the middle dilution should correspond approximately to the Bethesda Units of the plasma.

C. Factor VIII Inhibitor Free Plasma: To detect or exclude a very weak Factor VIII inhibitor the Factor VIII Inhibitor Free Plasma should be tested undiluted and at a dilution of 1:2 as a negative control (without Factor VIII inhibitor).

D. Normal Value: The comparison mixture (0.2 mL Imidazole buffer + 0.2 mL Factor VIII Normal Plasma) serves as reference value for the calculation of the residual % Factor VIII activities of each sample. It should be determined every time.

E. Performance of the Test: see under Point MANUAL / 3.Test

F. Assessment: Normally only one or two dilutions of every sample will fall within the range of the graph. In the case of two dilutions, the results should be averaged. Results with Bethesda Units/mL ≤ 1 should be regarded with caution. (see C).

Example: plasma dilution 1:8
Factor VIII-content of the incubated sample: 16.5%
Normal value: 47%
F VIII Residual Activity: 35% = 1.5 U.F VIII Inh./mL
Bethesda units in the test sample: 1.5 x 8 = 12 BU/mL

STANDARDISATION

The Coagulation Reference is calibrated against the plasma standard of the WHO.

LIMITATION OF THE TEST

Other inhibitors, for example a F IX inhibitor, can affect the F VIII deter-mination.
A new calibration is required for each batch of reagents where a calibration curve is necessary 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.

PRECISION

Reproducibility was determined with different samples.
The following results were obtained for day to day precision:

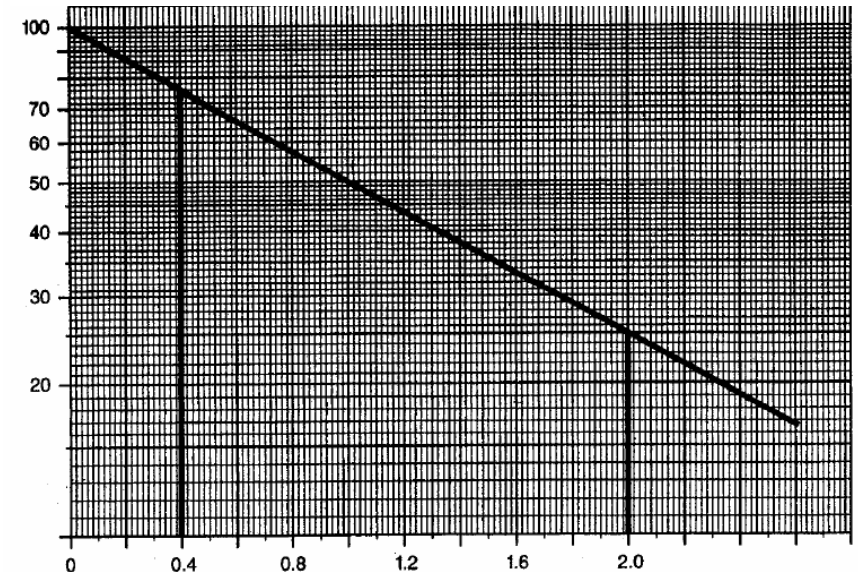
Sample	Sample 1	Sample 2
n	8	20
MV BU	1,7	19,9
SD	0,2	1,9
CV (%)	9,77	9,89

LITERATURE

Carol K. Kaspar et. al.: A More Uniform Measurement of Factor VIII Inhibitors ; Thrombos. Diathes. Haemorh. 34 (1975), 869

Graph

% F VIII-residual activity



U. F VIII Inh./mL