






Factor VIII Inhibitor Reagent Kit

For Research Use Only



REF 5152005 Factor VIII Inhibitor Reagent Kit (Bethesda Units)



Symbols key / Symbolschlüssel / interpretazione dei simboli / explicación de símbolos / Explicação dos símbolos / clé des symboles		
	manufactured by / Hergestellt von / prodotto da / fabricado por / fabricado por / fabriqué par	AQUA distilled water / destilliertes Wasser / acqua distillata / agua destilada / água destilada / eau distillée
	expiry date / Verfallsdatum / data di scadenza / fecha de caducidad / data de validade / date d'expiration	DIL dilute or dissolve in / verdünnen oder lösen in / diluire o dissolvere in / diluir o disolver / diluir ou dissolver em / diluer ou dissoudre dans
	storage temperature / Lagertemperatur / temperatura di conservazione / temperatura de conservación / temperatura de conservação / température de stockage	
	consult instructions for use / Gebrauchsanweisung beachten / consultare le istruzioni per l'uso / consulte las instrucciones de uso / consultar o manual de instruções / instruction d'utilisation	LOT lot / Charge / lotto / lote / lote / lot
CE	CE-mark / CE-Zeichen / marchio CE / marca de CE / Simbolo da CE / marquage CE	REF catalogue number / Katalognummer / numero di catalogo / número de catálogo / número de referência / réf. de catalogue
	determinations / Bestimmungen / determinazioni / determinaciones / determinações / déterminations	



PRODUCT DESCRIPTION

INTENDED 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
-	Calibration Plasma		
	[REF] 5220110	Coagulation Reference	5 x 1 mL

WARNING AND PRECAUTIONS

- 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

- 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).
- Normal value (comparison mixture):** The normal plasma is diluted with equal parts of Imidazole buffer in a similar way to the test sample.
- Test:** After incubating the samples for 2 hours at 37°C the Factor VIII value is determined.

- 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%.

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

- 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.

- 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:

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

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: Each mixture has to be incubated for exactly two hours in a water bath at 37°C and stored at 2...8°C 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).

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: Patient 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 determination.

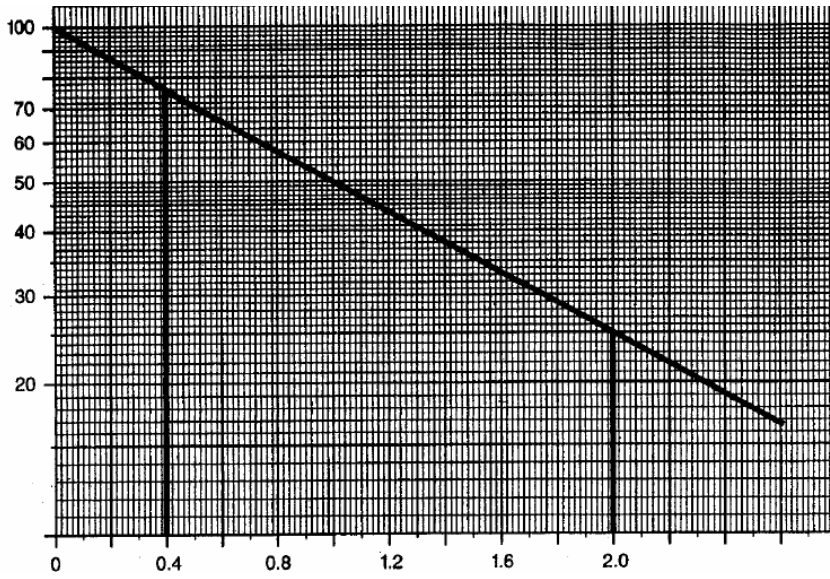
A new calibration is required for each batch of reagents where a calibration curve is necessary and for each instrument used. Also a new calibration is recommended, if software changes are introduced or following a major service of either instruments or equipment.

LITERATURE

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

Graph / Grafische Darstellung / Rappresentazione grafica / Presentación grafica / Représentation graphique

% F VIII-residual activity
% F VIII-Restaktivität
% attività residua del F VIII
% F VIII actividad residual
% d'activité résiduelle du F VIII



U. F VIII Inh./mL
E. F VIII Inh./mL
U. d'ini. del F VIII/mL
U. F VIII Inh./mL
U. inh. du F VIII/mL