# Factor VIII Inhibitor Reagent Kit

For Research Use Only

| Symbols key / Symbolschlüssel / interpretazione dei simboli / explicación de símbolos / Explicação dos símbolos / clé des symboles |  |
| manufactured by / Hergestellt von / prodotto da / 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 stockage |  |
| consult instructions for use / Gebrauchsanweisung beachten / consultare le istruzioni per l’uso / consultar las instrucciones de uso / consultar o manual de instruções / instruction d'utilisation | LOT | lot / Charge / lotto / lote / lote / lot |
| CE-mark / CE-Zeichen / marchio CE / marca de CE / Símbolo da CE / marquage CE | REF | catalogue number / Katalognummer / numero di catalogo / número de catalago / número de referência / ref. de catalogue |
| determinations / Bestimmungen / determinazioni / determinaciones / determinações / déterminations |  |
**PRODUCT DESCRIPTION**

**INTENDEN USE**
Reagent kit for carrying out Factor VIII inhibitor assays.

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

- 2 x Factor VIII Normal Plasma = 3 mL
- 1 x Factor VIII Inhibitor Plasma 1 mL
- 1 x Factor VIII Inhibitor Free Plasma l.a.d.s. 1 mL
- 1 x Imidazole buffer, sterile 17 mL

The reagents for Factor VIII assay are not included.

**MATERIAL REQUIRED**

- Pipettes
- Distilled water
- Solutions/buffers:
  - CaCl₂, 25 mmol/L, Solution 100 mL
  - Imidazole Buffer 50 mL
- Reagents
  - F VIII Deficient Plasma, native 5 x 1 mL
  - Daptin TC 5 x 2 mL
  - Daptin TC 6 x 10 mL
- Chromogenic method
  - Technochrom F VIII:C 40 tests
- Calibration Plasma
- Coagulation Reference 5 x 1 mL

**STABILITY AND STORAGE**

- 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).

**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 l.a.d.s., 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**

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:** After incubating the samples for 2 hours at 37°C the Factor VIII value is determined.

**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).

**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 can be used. For 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 can be used. For F VIII method (one stage method: 1:5 with imidazole buffer dilution, TECHNOCHROM F VIII:C 1:41 with F VIII dilution buffer).

**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.

**LITERATURE**
Carol K. Kaspar et. al.: A More Uniform Measurement of Factor VIII Inhibitors ; Thrombos. Diathes. Haemorrh. 34 (1975), 869
% F VIII-residual activity
% F VIII-Restaktivität
% attività residua del F VIII
% F VIII actividad residual
% d’activité résiduelle du F VIII