ROX FIX-A – 95 00 30

For In Vitro Research Use – Not For Diagnostic Use

ENGLISH – Insert Revision 04/2014

1 INTENDED USE

For quantitative determination of human Factor IXa (FIXa) activity as contamination in human Factor IX (FIX) containing concentrates.

2 BIOCHEMISTRY

FIX is a single chain, vitamin K dependent glycoprotein of about 55 KDa, which is activated by FXIa or tissue factor / FVIIa. Activated FIX (FIXa) converts FX to FXa in the presence of FVIII, phospholipids and calcium ions.

3 MEASUREMENT PRINCIPLE

FIXa activity in FIX containing concentrates is determined in a chromogenic method, in which human FX is activated by contaminating human FIXa in the FIX concentrate in the presence of FVIII, thrombin, calcium ions and phospholipid. The amount of generated human FXa is determined from the hydrolysis of a chromogenic FXa substrate. The sample FIXa activity is determined by the slope ratio model in which the potency of the sample is calculated vs. a FIXa standard with potency expressed in International Units (IU).

4 KIT COMPOSITION

Reagent 1 (2 vials) – REF 9510
Reagent 1 contains lyophilized human Factor VIII and human FX.
Each vial is sufficient for 50 tests.
Reagent 2 (2 vials) – REF 9520
Reagent 2 contains lyophilized human thrombin, calcium chloride and phospholipids.
Each vial is sufficient for 50 tests.
FXa Substrate, 6 mL (1 vial) – REF 9080
Liquid solution of chromogenic FXa substrate (Z-D-Arg-Gly-Arg-pNA), 2.5 mmol/L, containing a thrombin inhibitor.
FIXa Diluent Buffer, Stock Solution, 20 mL (1 vial) – REF 9550
Liquid stock solution of diluent buffer.

5 PRECAUTIONS AND WARNINGS

The reagents are matched – only use reagents from the same kit lot.

CAUTION: Each donor unit used in the reagents has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and antibodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood borne diseases, the handling and disposal of these human sourced reagents should be handled with the required caution, as being potentially infectious.

6 PREPARATION

Reagent 1
Reconstitute with **2.8 mL** water. Allow to stand for 5 min at 20-25°C with intermittent gentle mixing for complete reconstitution.
Reagent 2
Reconstitute with **4.0 mL** water. Allow to stand for 5 min at 20-25°C with intermittent gentle mixing for complete reconstitution.
FXa Substrate, 6 mL
Ready for use.
Diluent Buffer, Stock Solution, 20 mL
Before use, dilute 1 + 9 with water to obtain a 0.05 mol/L Tris-HCl buffer working solution, pH 7.5 (at 20°C) with 1% bovine serum albumin.

Note: All reconstitutions and dilutions should be made with water of a quality of at least NCCLS Type II water or Ph. Eur. water for injection.

7 STORAGE AND STABILITY

The sealed reagents are stable at 2-8°C until the Expiry Date printed on the label. Be careful to avoid contamination of the reagents by microorganisms.

- **Reconstituted Reagent 1 and Reagent 2**:
  Stability after reconstitution is 48 hours at 2-8°C.
- **Chromogenic FXa substrate**:
  Opened vial is stable for 1 month at 2-8°C.
- **FIXa Diluent Buffer**:
  Stock Solution: Opened vial is stable for 1 month at 2-8°C.
  Buffer working solution should be used the same day as prepared.

8 Calibrator and Control

FIXa Calibrator (REF 9599) and FIXa Control (REF9588) are available from Rossix. Both products are potency assigned vs. a WHO International Standard for FIXa.

9 MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized water, NCCLS Type II water or Ph Eur water for injection or higher quality.
- Citric acid, 2% (for end-point method)
- Calibrated pipettes
- Photometer, 405 nm (and 490 nm for end-point method)
- Heat incubator 37°C
- Plastic test tubes
- Vortex mixer
- Stop-watch
- FIXa Calibrator and Control

SYMBOLS USED

Catalogue number

Batch code

Use by

Temperature limitation


Consult instruction for use


Biological risks


Manufacturer

10 HEPARIN NEUTRALIZATION

For samples containing heparin, neutralization of heparin activity should be made by use of e.g. the heparin antagonist PolyBrene®. A suitable stock solution of Polybrene is available from Rossix upon request.

11 METHOD

11.1 Standard dilutions

A standard curve should be included in each run.
Prepare standard dilutions in FIXa Diluent Buffer working solution to obtain standards in the range 0.02–0.8 mIU/mL. Prepare at least five different standard dilutions. All dilutions should be prepared in plastic test tubes.

Example:
Predilute the FIXa Calibrator to a potency of 1.0 mIU/mL and prepare further dilutions according to the table below.
It is recommended to prepare independent predilutions for each standard.

Preparation of FIXa Standard dilutions, RANGE 0.02 – 0.8 mU/mL		
FIXa Standard mIU/mL	**Volume of FIXa Standard**	**Volume of Diluent Buffer working solution**
0.8 (100%)	**300 µL** of 1 mIU/mL FIXa	+ **75 µL**
0.56 (70%)	**300 µL** of 1 mIU/mL FIXa	+ **236 µL**
0.32 (40%)	**300 µL** of 1 mIU/mL FIXa	+ **638 µL**
0.08 (10%)	**100 µL** of 1 mIU/mL FIXa	+ **1150 µL**
0.02 (2.5%)	**50 µL** of 1 mIU/mL FIXa	**+2450 µL**
0	-	**500 µL**

NOTE: The above table is an example only and is based on a FIXa calibrator with an activity of 1.0 mIU/mL. The volumes should be adjusted for the potency of the calibrator being used.

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11.2 Sample dilution

It is recommended to analyse the FIX concentrate sample at several different dilutions, starting at a FIX potency of 1.0 IU/mL, to establish the minimal dilution required to obtain a linear dose-response. For subsequent analysis only use those sample dilutions that fulfil the requirement of a linear dose-response. The FIXa activity assignment of the tested FIX concentrate should then be determined according to the slope ratio model (see 13 Calculations).
All dilutions should be prepared in plastic test tubes with FIXa Diluent Buffer working solution as diluent.

Example:
Assume a linear dose-response is obtained from a FIX potency of 0.5 IU/mL. Set 0.5 IU/mL = 100%

Factor IX Sample Dilutions		
Sample	**Volume of FIX Sample**	**Volume of Diluent Buffer working solution**
100%	**1000 µL** of 0.5 IU/mL	0 **µL**
70%	**300 µL** of 100% sample	+ **129 µL**
40%	**300 µL** of 100% sample	+ **450 µL**
10%	**300 µL** of 100% sample	+ **2700 µL**

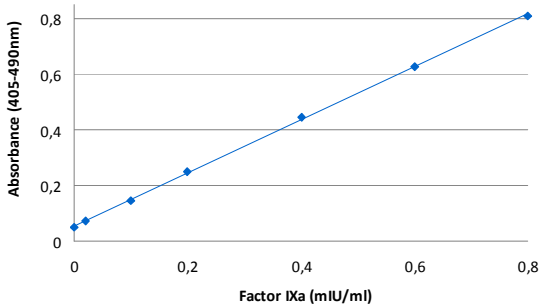
NOTE: The above table is an example only.

11.3 Assay

Sample / Standard dilution (20-25°C)	**50 µL**
Reagent 1 (20-25°C)	**50 µL**
Incubation 2-4 min, 37°C	
Reagent 2 (37°C)	**75 µL**
Activation 4 min, 37°C	
FXa Substrate (37°C)	**50 µL**
Kinetic method: Read ΔA405/min at 37°C *End-point method: Incubate at 37°C for 20 min*	
Citric Acid, 2% (End-point method only)	**50 µL**

Kinetic method:
Read the absorbance change at 405 nm (ΔA405/min). A lag time may be introduced before starting the data acquisition.
End-point method:
Read the absorbance at 405 nm, using 490 nm as reference wavelength. Absorbance readings should be made within 4 hours.

12 TYPICAL STANDARD CURVE



The above graph is an example only. A standard curve should be included in each run.

13 CALCULATION

13.1.1 Slope ratio model

The European Pharmacopoeia recommends the use of the slope ratio or parallel line model.[1]

1. Plot the maximal absorbance change/minute ($\Delta A405_{max}$/min) or absorbance (A405-490) vs. FIXa activity (mIU/mL or %) in a Lin-Lin graph
2. Assign the sample FIXa activity from the standard curve using the slope ratio model.
3. Adjust for the dilution and express the results as mIU FIXa/ml or mIU FIXa/IU FIX.

13.1.2 Potency assignment directly from the calibration curve.

As an alternative to the slope ratio model, the FIXa activity in the sample dilution can be directly obtained from the calibration curve. The result should then be multiplied by the dilution factor used.

NOTE: If the direct method is used it is important to verify that the analysed sample is tested at a dilution which is within a dilution range that gives a proper linear dose-response. If not, there is a risk of underestimation of the FIXa activity in the sample.

14 CONTACT INFORMATION

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15 REFERENCES

1. 6th Edition of the European Pharmacopoeia, General Chapter 5.3 Statistical analysis of results of biological assays and tests.
2. Gray E, Tubbs J, Thomas S et al. Measurement of activated Factor IX in Factor IX concentrates: Correlation with In Vivo thrombogenicity. *Thromb Haemost 1995*; **73** (4): 675-679.
3. Pickering WM, Gray E. The effect of activated Factor IX on the Factor IX coagulant and NAPTT activity of high-purity Factor IX concentrates. *J Thromb Haemost 2007*; **5**, Supplement 2: P-T-156.
4. Böhm E, Dockal M, Pilz J, Schrenk G, Varadi K, Scheillinger F. Superiority of the chromogenic assay specific for activated factor IX (FIXa) over the non-activated partial thromboplastin time (NAPTT) clotting assay in detecting FIXa in recombinant FIX (rFIX) preparations. *XXIV ISTH Congress 2013; Poster PB1.39-6.*