Chromogenic Assay

FACTOR VIII

1. TECHNOCHROM® FVIII:C

**Kit contents:**
- 2 vials of Substrate FXa-1+αNAPAP (2 mL)
- 2 vials of Reagent A (PL, Albumin) (2 mL)
- 2 vials of Reagent B (Factor IXaβ, Factor X, Ca++, Albumin, Thrombin) (2 mL)
- 1 vial of Ref. Stand. FVIII 1 (=130%) (1 mL)
- 1 vial of Ref. Stand. FVIII 2 (=70%) (1 mL)
- 1 vial of Ref. Stand. FVIII 3 (=10%) (1 mL)
- 1 vial of Ref. Stand. FVIII 4 (<0.5%) (1 mL)
- 3 bottles of Dilution buffer (30 mL)
- 2 vials of Reaction buffer (8 mL)

**Reagent kit for photometric FVIII:C determinations**

TECHNOCHROM® FVIII:C 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. The kit shows an excellent correlation with one and two stage Factor VIII assays and a linear calibration curve between 0 and 130%. It contains all components and is insensitive to heparin up to 10 IU/mL.

**Use**
- Linearity: 0 – 130 (% activity).
- Limit of detection: 0 % (activity %)
- 5 minutes incubation and reading the results over 3 minutes.

**Features & benefits**
- Stability on board 24 hours
- Reagents may be capped and refroze 2 weeks at -20°C after opening.

**Related products**
- Coagulation Reference
- Coagulation Control N
- Coagulation Control A

**Example: Manual method (kinetic and end-point method)**

![Graph showing kinetic and endpoint method](image)
FACTOR ASSAYS

Chromogenic Assay

FACTOR IX

1. ROX FACTOR IX

Kit contents:
- 2 vials of Reagent A (Human Factors VIII and X, bovine Factor V, and a fibrin polymerization inhibitor).
- 2 vials of Reagent B (Human Factors XIa and II, calcium chloride and phospholipids)
- 1 vial of Chromogenic FXa Substrate, 6 mL
- 1 bottle of Dilution buffer, 20 mL

Kit for determination of Factor IX activity in plasma and Factor IX preparations, including potency assignment of FIX concentrates.

FIX is activated by human FXIa with concomitant activation of human FX by generated FIXa in the presence of FVIII, calcium ions and phospholipids. The amount of formed FXa is related to the FIX activity and is measured through hydrolysis of a chromogenic FXa substrate. There is no use of FIX deficiency plasma. The Kit contains a fibrin polymerization inhibitor and a heparin antagonist.

Features & benefits
- There is no interference of FIXa up to 50 mIU FIXa/1 IU FIX.
- No use of FIX deficiency plasma
- Detection Limit: 0.1 % FIX (CLSI EP17-A)
- Quantification Limit: 0.5 % FIX (CLSI EP17-A)
- Linearity: 0.5 – 200 % (CLSI EP06-A)

Characteristics

FIX activity is determined in a chromogenic method, in which human FIX is activated by human FXIa and where formed FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. Similar to in vivo conditions, FVIII is activated by thrombin which is generated during the incubation. The amount of FXa formed is related to the FIX activity and is determined from the hydrolysis of a chromogenic FXa substrate. The FIX activity of the sample is assigned vs. a FIX plasma or a FIX concentrate standard with FIX potency expressed in International Units (IU).
Chromogenic Assay

FACTOR IXa

1. ROX FIX-A

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
<th>Package size</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-950030</td>
<td>kit</td>
<td>2 x 50</td>
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</tbody>
</table>

Kit contents:
- 2 vials of Reagent 1 (Human Factors VIII and X)
- 2 vials of Reagent 2 (Human Thrombin, CaCl₂ and phospholipids)
- 1 vial of chromogenic FXa Substrate, 6 mL
- 1 bottle of FIXa Diluent Buffer, Stock Solution, 20 mL

A chromogenic kit for quantitative determination of human Factor IXa (FIXa) activity as contamination in human Factor IX (FIX) containing concentrates.

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).

2. FACTOR IXa CALIBRATOR

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9599</td>
<td>10 x 2.0 mL</td>
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</table>

Factor IXa Calibrator intended for use with the Rox FIX-A kit.

Lyophilized preparations of human Factor IXa in packages of 10 x 2 mL. Factor IXa Calibrator is calibrated against a WHO Factor IXa International Standard.

3. FACTOR IXa CONTROL

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
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</thead>
<tbody>
<tr>
<td>5-9588</td>
<td>10 x 2.0 mL</td>
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</tbody>
</table>

Factor IXa Control intended for use with the Rox FIX-A kit.

Lyophilized preparations of human Factor IXa in packages of 10 x 2 mL. Factor IXa Control is calibrated against a WHO Factor IXa International Standard.

Features & benefits:
- Much better detection limit (0.005%) than NAPTT method.
- Quantification limit = 0.02 mIU/ml FIXa.
- Detects a preactivation level in the order of 0.002%.
- Reconstituted reagents are stable for 48 h at 2-8°C.
- Factor IXa Calibrator and Control available.

Characteristics

The kit method comprises activation of human FX by sample FIXa in the presence of FVIII, thrombin, calcium ions and phospholipids. Generated FXa is directly proportional to the FIXa activity in the sample and is measured through hydrolysis of a chromogenic FXa substrate.
FACTOR ASSAYS

Chromogenic Assay

FACTOR XIa

1. ROX FACTOR XIa

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
<th>Package size</th>
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<tbody>
<tr>
<td>5-110050</td>
<td>kit</td>
<td>2 x 50</td>
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</tbody>
</table>

Kit contents:
- Reagent 1 (2 vials) – REF 1110
- Reagent 2 (2 vials) – REF 1120
- FXa Substrate, 6 mL (1 vial) – REF 9080
- Diluent Buffer, Stock Solution, 20 mL (1 vial) – REF 1150

A chromogenic kit for quantitative activity determination of Factor XIa in enriched or highly purified protein preparations.

Human FXIa is determined from its activation of human Factor IX and ensuing activation of human Factor X. Generated Factor Xa is then measured with a chromogenic FXa substrate. The activation of Factor IX is performed in two steps: Initial activation of FIX in the absence of phospholipids and continued activation of FIX in the presence of FX and phospholipids, therewith allowing concomitant activation of FX.

Features & benefits
- Detection Limit = about 0.03 mU/mL
- No use of human plasma

2. FACTOR XIa CALIBRATOR

<table>
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<th>Cat. number</th>
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<tr>
<td>5-1199</td>
<td>10 x 4.0 mL</td>
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</tbody>
</table>

Factor XIa Calibrator intended for use with the Rox Factor XIa kit.

Lyophilized preparations of human Factor XIa in packages of 10 vials. The Factor XIa Calibrator is calibrated against the NIBSC reference reagent for Activated Blood Coagulation Factor XI (FXIa), 11/236, and potency assigned in Units/mL.

3. FACTOR XIa CONTROL

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-1188</td>
<td>10 x 4.0 mL</td>
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</table>

Factor XIa Control intended for use with the Rox Factor XIa kit.

Lyophilized preparations of human Factor XIa in packages of 10 vials. The Factor XIa Control is calibrated against the NIBSC reference reagent for Activated Blood Coagulation Factor XI (FXIa), 11/236, and potency assigned in Units/mL.
FACTOR ASSAYS

Chromogenic and ELISA Assays

FACTOR XIII

CHROMOGENIC ASSAYS

1. TECHNOCHROM® FXIII

DEFICIENT PLASMA

2. Factor XIII Deficient Plasma

ELISA ASSAY

3. TECHNOZYM® FXIII Ag ELISA

4. TECHNOZYM® FXIII-A Sub ELISA

5. TECHNOZYM® FXIII-B Sub ELISA

Avantages

The method is linear up to 300 % FXIII activity.
The detection limit is 0.6 % with blank reagent included.
"When a blank reagent is not supplied by the manufacturer, the limit of quantification is between 3 % and 5 %.”

H. P. KOHLER and al, Diagnosis and classification of factor XIII deficiencies, journal of Thrombosis and Haemostasis, 9: 1404–1406 July 2011

Lawrie et al, J Thrombosis and haemostasis 2010;8: 2478-82

Related products

Coagulation Reference  Coagulation Control N  Coagulation Control A

1. TECHNOCHROM® FXIII

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
<th>Package size</th>
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</thead>
<tbody>
<tr>
<td>4-5360010</td>
<td>kit</td>
<td>3 x 20</td>
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</tbody>
</table>

Kit contents:
- 3 vials of Activator Reagent (3 mL)
- 3 vials of Detection Reagent (3 mL)
- 3 vials of NADPH Solution (3 mL)
- 3 vials of Inhibitor Reagent (1 mL)
- 1 vial of Stabilizer Solution (6 mL)

Reagent kit for the determination of blood coagulation Factor XIII (FXIII) activity to detect inherited or acquired FXIII deficiencies, abnormal FXIII with decreased activity and elevated FXIII level.

FXIII present in the plasma sample is activated by thrombin and Ca²⁺. The formed FXIIIa then cross-links the amine substrate glycine ethyl ester (GEE) to the glutamine residue of specific peptide substrate P1(1-12), and ammonia is released. In the indicator reaction the amount of released ammonia is monitored in a glutamate dehydrogenase catalysed NADPH-dependent reaction. The consumption of NADPH is measured spectrophotometrically by the decrease of absorbance at 340 nm. Within a time window the decrease of absorbance is directly proportional to the FXIII activity. This assay can be used for monitoring FXIII replacement therapy.

Information

Inherited FXIII deficiency is a rare, but occurs with severe bleeding diathesis with occasional wound healing impairment and in women with habitual abortion.

2. FACTOR XIII DEFICIENT PLASMA

<table>
<thead>
<tr>
<th>Cat. number</th>
<th>Conditioning</th>
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</thead>
<tbody>
<tr>
<td>4-5194104</td>
<td>5 x 1.0 mL</td>
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</tbody>
</table>

Factor XIII deficient plasma (immune-adsorbed) used as negative control in FXIII assays.

The Factor XIII deficient plasma is an immune-adsorbed lyophilised, stabilised human plasma with a Factor XIII content of <1 %, whereas all the other coagulation factors are within about the normal range.
3. TECHNOZYM® FXIII AG ELISA

Kit contents:
- 12 ELISA test strips (12 x 8 wells)
- 1 vial of Capture antibody
- 1 bottle of Wash solution, concentrate (100 mL)
- 1 bottle of Sample Dilution buffer (100 mL)
- Calibrators, lyophilised; 1 vial each (0.25 mL)
- Control plasmas high and low level; 1 vial each (0.25mL)
- 2 bottles of TMB (12 mL)
- 1 bottle of Stop solution (12 mL)
- Adhesive film, 2 pieces.

Antigene ELISA test used for determination of blood coagulation factor XIII (FXIII) in human plasma.

The high sensitivity of the assay allows the quantitative measurement of the extremely low plasma Factor XIII (FXIII) levels that might occur in congenital FXIII deficiency. Only the FXIII complex is measured, the presence of free subunits does not interfere with the assay. The TECHNOZYM® FXIII Ag ELISA kit is a one-step sandwich enzyme immunoassay using monoclonal antibodies specific to FXIII-A and FXIII-B subunits. Plasma FXIII is a heterotetramer (FXIII A2B2) consisting of A (FXIII-A) and B (FXIII-B) subunits. With the exception of the extremely rare inherited FXIII-B deficiency, all A subunits are complexed with B subunits in plasma, while about 50 % of the B subunits exist in free form.

4. TECHNOZYM® FXIII-A SUB ELISA

ELISA test used for determination of FXIII-A subunit in human plasma and in cell lysates.

Plasma FXIII is a heterotetramer (FXIII A2B2) consisting of two potentially active A Subunits (FXIII-A) and two carrier/inhibitory B Subunits (FXIII-B). All A subunits are complexed with B subunits in plasma, while about 50 % of the B subunits exist in free form. Free A-subunits only exist in the extremely rare inherited FXIII-B deficiency. Inherited deficiency of FXIII-A or autoantibody directed against FXIII-A causes severe bleeding diathesis with high risk of intracranial bleeding. In women who lack FXIII-A, pregnancy cannot be maintained.
**1. ROX FACTOR PROTHROMBIN**

**Chromogenic Assay**

**PROTHROMBIN**

1. **ROX Factor Prothrombin**

Kit contents:
- Activator Reagent, 3.0 mL (4 vials) – REF 2010
- FIIa Substrate, 6 mL (1 vial) – REF 2080
- FII Diluent Buffer, Stock Solution, 20 mL (1 vial) – REF 2050

Kit for quantitative determination of Prothrombin (FII) functional activity in plasma and FII containing concentrates. The method is suitable for plasma collected in citrate or EDTA.

FII functional activity is determined in a chromogenic prothrombinase method, in which human FII is activated to thrombin (FIIa) by human FXa in the presence of bovine FVa, calcium ions and phospholipid.

The amount of FIIa formed is determined from the hydrolysis of a chromogenic FIIa substrate. The FII activity of the sample is assigned vs. plasma or a FII concentrate standard with FII potency expressed in International Units (IU).

The prothrombinase complex is sensitive to γ-carboxylation and therefore non-γ-carboxylated-FII is not activated in this method in contrast to snake venom based prothrombin methods.

**Features & benefits**

Factor II results are not affected at plasma levels up to the stated levels below:
- Hemoglobin: 10 mg/mL
- Bilirubin: 800 μg/mL
- Triglycerides: 10 mg/mL
- Heparin, LMW: 4 U/mL
- Heparin, UF: 4 U/mL

**Characteristics**

- A chromogenic Prothrombin method based upon the prothrombinase complex.
- non-γ-carboxylated-FII is not activated in the method in contrast to snake venom based prothrombin methods.
- Detection Limit = about 0.05 U/mL (5 %) when using a plasma dilution of 1:200 as prescribed in the package insert. The detection limit for undiluted samples is about 0.25 mU/mL.

**Information**

Factor II is a single chain vitamin K dependent glycoprotein of 72 kDa, which is activated to thrombin (FIIa) by FXa in the presence of FVa, calcium ions and phospholipids.