CE XXXX IVD

Precision in Hemostasis

Behnk Elektronik

BE Factor IX Deficient Plasma FIX

Auxiliary reagent (human citrated plasma) for the quantitative determination of Factor IX activity in human citrated plasma

REF 771609: DP (6 x 1 mL)

| INTENDED USE

Freeze dried citrated human plasma from which Factor IX has been removed by selective immuno-adsorption.
Suitable for use with manual procedure on semi-automated coagulometer, or on automated coagulation analyzer, it is an auxiliary reagent for the *in vitro* quantitative determination of Factor IX in human citrated plasma using BEHNK reagents for Activated Partial Thromboplastin Time (APTT) measurements as follows:
REF 775100: BE Cal Ref (Reference plasma for calibration)
REF 771200, REF 771201: BE APTT K Kaolin + CaCl
REF 771250, REF 771251: BE APTT SL Silica + CaCl
REF 771700: BE Owren Buffer (Plasma dilution buffer)
The assay is intended as an aid in the laboratory investigation of coagulation abnormalities and for characterization of coagulation factor activity. Results must be interpreted in conjunction with clinical findings and other laboratory tests. For clinical laboratory use by healthcare professionals.

PRINCIPLE [1]

The test is based on the measurement of clotting time in the presence of cephalin and activator with a method in which all factors are present in excess (supplied by Factor IX Deficient Plasma) except Factor IX, which is derived from the sample to be tested.

GENERALITIES [2] [3] [4] [8] [9] [11] [15]

Factor IX is a glycoprotein synthetized by the liver. The synthesis of biologically active FIX (carboxylate) is vitamin K dependent.
Then, the fixation of activated Factor IX on platelets or tissue phospholipids is possible in the presence of Ca^{2+}.
Factor IX may be activated in two different ways:

- In the presence of Ca^{2+}, Factor Xa activates FIX to FIXa.
- Tissue factor/FVIIa complex activates either FIX or FX.

FIXa forms an enzymatic complex with phospholipids, Ca^{2+} and FVIIIa; this complex then activates Factor X to Factor Xa.

Decrease of FIX activity is associated with:

- Hemophilia B:

The seriousness of hemophilia is assessed on the basis of the concentration of FIX:C.

Severe hemophilia	< 1 %
Moderate hemophilia	1 to 5 %
Hemophilia attenuated	5 to 25 %

- Hypovitaminosis K:
  - AVK Treatment
  - Nutritional intake deficiency, disorders in absorption or metabolism of vitamin K (hemorrhagic disease of the newborn, cholestasis, treatments with antibiotics)
- Liver diseases:
  - Cirrhosis
  - Hepatitis
- Decrease of the level of FIX in the presence of FIX inhibitor.

REAGENTS

DP **FIX** Deficient Plasma FIX

Freeze dried human citrated plasma

According to (EC) 1272/2008 regulation, this reagent is not classified as dangerous.

SAFETY CAUTIONS [11] [12]

- Refer to Safety Data Sheet (SDS) available on request or on www.behnk.de
- Each donor unit used to manufacture this product was tested and found non-reactive for HbsAg, antibody to Hepatitis C and antibody to HIV-1/HIV-2.
- However, no test method can offer complete assurance that infectious agents are absent. All specimens or reagents from biological origin should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions.
- Waste disposal: Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

PREPARATION OF REAGENTS

Open the vial carefully.
Add exactly the volume of demineralized water stated on the label.
Cap the vial and let stand for 15 minutes at room temperature.
Mix gently by swirling and inverting before use, to homogenize the content.

STABILITY AND STORAGE

Unopened vials, stored away from light at 2-8 °C are stable until the expiry date stated on the label.
Stability after reconstitution:

- 18-25°C 4 hours
- On board Stability (OBS)* 4 hours

* 16-22 °C

Do not use any reagent after expiry date.

SAMPLES COLLECTION AND HANDLING [13] [14]

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (trisodium citrate anticoagulant solution 0.109 M, *i.e.* 3,2%). Mix immediately the blood with anticoagulant.
Avoid drawing with a syringe that could result in the formation of micro-clots.
Centrifuge for 15 minutes at 2000-2500 g and extract supernatant.
Storage in plastic tube: 4 h at 15-25°C, 15 days at -20°C*, 1 month at -80°C*.
*If quickly frozen; thaw plasmas at 37°C until complete thawing.

Caution: if the same plasma is used for testing Factor VII, do not store at 2-8°C, because the Factor VII may be activated by the kallikrein system in this temperature range.

LIMITS [6] [7]

Heparins and Thrombin inhibitors (hirudin, argatroban, …) present in the specimen may decrease Factor IX activity in the specimen.
The presence of Lupus anticoagulants may lead to an underestimation of Factor IX activity in the specimen.
For a more comprehensive review of factors affecting this assay, refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

Basic medical analysis laboratory equipment
Automated or semi-automated coagulation analyzer
Demineralized water

REFERENCE RANGE [2]

Plasma (adult): Usually 60-150 %

Each laboratory should establish its own normal ranges for the population that it serves.

QUALITY CONTROL

REF 773100: BE Trol 1; REF 773101: BE Trol 2
Controls are required for checking the accuracy and reproducibility of the results.
The control intervals should be adapted to each laboratory's individual requirements.
Values obtained should fall within the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

PROCEDURE

Manual method on semi-automated systems:
Pre-incubate CC reagent ($CaCl_2$ 0.025 M) of the APTT reagent 15 min to reach a temperature of 37 °C.
Dilute specimens and controls: 1/10 in BE Owren Buffer.
Calibrators: prepare dilutions as indicated in § Calibration:

- Diluted specimen (calibrators, controls, plasmas): 100 µL
- Deficient Plasma: 100 µl
- APTT reagent (mix before use) 100 µl

Incubate for 180 sec at 37 °C

- CC reagent (37 °C): 100 µL

The automatic countdown timer will start immediately after CC reagent addition and stop when the clot is formed.

Automated method on Behnk Thrombolyzer series:
Refer to the full detailed application specific to the automated system.

Note:

- Performances and stability data have been validated on Thrombolyzer Compact X (available on request). These data are valid for all instruments in the Thrombolyzer series.
- With manual procedure and on other automated coagulation analyzer, performances and stability data must be validated by user.

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CALIBRATION [12]

Use REF 775100: BE Cal Ref

Reference plasma traceable to SSCLOT5, non WHO SSC/ISTH Secondary Coagulation Standard from NIBSC.

Manual method on semi-automated analyzer: Prepare a calibration curve with dilution 1/10, 1/20, 1/40 and 1/80 of reference plasma in BE Owren Buffer.

Measure in triplicate the clotting time of each level.

Automated method on Behnk Thrombolyzer series: Perform a calibration with BE Cal Ref using automatic dilutions indicated in the specific application.

CALCULATION

Results are expressed in % of Deficient Factor according to the calibration curve.

PERFORMANCES

The studies were performed on Thrombolyzer Compact X.

Precision: Repeatability (Within run) and Reproducibility (Between run)

Within run N = 20	Level 1	Level 2
Mean (%)	158	56
S.D. (%)	8.2	2.8
C.V. (%)	5.4	5.0

Between run N = 20	Level 1	Level 2
Mean (%)	132	47
S.D. (%)	9.4	3.3
C.V. (%)	7.1	7.0

Detection limit: equivalent to 6 % of Factor IX

Measuring Range: from 12 % (QL) to 200 %

Interferences (APTT Silica, sec):

Turbidity	No interference up to 731 mg/dL of Triglycerides
Bilirubin	Positive interference from 124 µmol/L
Hemoglobin	No interference up to 261 µmol/L

Other substances may interfere with the results (see § Limits)

Calibration Stability: Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

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(11) *SCHMIDT A.E., BAJAJ S.P.: "Structure-Function Relationships in Factor IX and Factor IXa". Trends Cardiovasc. Med., 13, 1, 39-45, 2003.*

(12) *Secondary Coagulation Standard Lot #5 NIBSC code: SSCLOT5 Instructions for use (Version 3.0, Dated 03/08/2020)*

(13) *CLSI Document GP41-A6: "Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard". Sixth edition, 27, 26, 2007.*

(14) *CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline". Fifth edition, 28, 5, 2008.*

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| = Significant modifications

IFU_771609-EN_V03_20260810

Table of symbols

Manufacturer	Expiry date	*In Vitro* Diagnostic	Temperature limitation	Catalogue number	See insert	Batch number	Store away from light	Sufficient for	Dilute with	Demineralized water	Biological hazard
		IVD		REF		LOT			→	H_2O	

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