CE IVD

Precision in Hemostasis

Behnk Elektronik

BE Factor IX Deficient Plasma FIX

Immuno-depleted plasma for the determination of Factor IX activity in human plasma

REF 771609: DP (6 x 1 mL)

| INTENDED USE

This reagent is designated for professional use in laboratory (semi-automated or automated method). It allows the quantitative determination of Factor IX activity in citrated human plasma to assess the status of coagulation factors normally found in blood.
This test is realized with Behnk reagents as follows:
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)

| 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] [7] [8]

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 (hemorragic 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 — Human Origin

Freeze dried citrated plasma without Factor IX, removed by selective immune adsorption.

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

| SAFETY CAUTIONS [11] [12]

- Refer to current Material Safety Data Sheet 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 and add exactly the volume of demineralised 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 homogenise 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:

- 2-8 °C 8 hours
- On board Stability (OBS)* 4 hours
- 15-25 °C 4 hours

* 18-22 °C

Do not use any reagent after expiry date.

SAMPLES COLLECTION AND HANDLING [9] [10]

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (sodium citrate solution 0.109 M). Mix immediately the blood with anticoagulant.
Avoid drawing with a syringe that could result in the formation of micro-clots.
Centrifuge for 10 minutes at 3000 g and extract supernatant.
Stability:

- 4 h at 2-25 °C
- 15 days at -20 °C, 1 month at -80 °C (if quickly frozen. Defrost at 37 °C until complete defrosting).

LIMITS [5] [6]

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
Demineralised water

REFERENCE RANGE [2] [7]

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

| 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).
- With manual procedure and on other automated coagulation analyzer, performances and stability data must be validated by user.
- Other validated applications or proposal applications are available on request.

Manufactured by
BIOLABO S.A.S.
Les Hautes Rives
02160 Maizy, France

Made in France

Distributed by
Kommanditgesellschaft Behnk Elektronik GmbH & Co.
Hans-Böckler-Ring 27
22851 Norderstedt, Germany

T. +49 (0)40-529 861 0
F. +49 (0)40-529 861 99
info@behnk.de

Latest revision: www.behnk.de

CALIBRATION

Use REF 775100: BE Cal Ref
Reference plasma traceable to WHO SSC/ISTH Secondary Coagulation Standard NIBSC code: SSCLOT4.
Semi-automatized method:
Prepare a calibration curve with dilution 1/10, 1/20, 1/40 and 1/80 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:

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.

REFERENCES

(1) *SOULIER J.P., LARRIEU M-J.: Sang. 24, 3, 205-215, 1953*
(2) *CAEN J., LARRIEU M-J., SAMAMA M.: Paris, L'Exp. Scient., 181, 1975*
(3) *ORSTAVIK K.H., LAAKE K.: "Fator IX in wrafarin treated patients". Thromb.RES., 13, 2, 207-218, 1978*
(4) *PANICUCCI F., SAGRIPANTI A. CONTE B PINORI E., VISPI M., LESCHINI L.:"Characterization of heterogeneity of haemophilia B for detection of carriers". Haemostasis, 9, 310-318, 1980.*
(5) *BRANDT J.T., TRIPLETT D.A., ROCK W.A., BOVILL E.G., ARKIN C.F.: "Effect of lupus anticoagulants in activated partial thromboplastin time". Arch. Pathol. Lab. Med., 115, 109-114, 1991*
(6) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-254 à 3-257*
(7) *SAMPOL J., ARNOUX D., BOUTIERE B.: "manuel d'hemostase" Paris: Editions scientifiques et médicales ELSEVIER, 311-336, 379-381, 552-553, 608-609, 1995.*
(8) *WHITE G.C., ROSENDAAL F., ALEDORT L.M., LUSHER J.M., ROTSHILD C., INGERSLEV J.: "Definition in haemophilia-Recommendation of the Scientific Subcommittee on factor VIII and factor VIII of the Scientific and Standardization Committee of the international society on Thrombosis and haemostasis"Thromb. Haemostasis, 85, 560, 2001*
(9) *WOODHAMS B., GIRARDOT O., BLANCO M.J., COLESSE G., GOURMELIN Y.: "Stability of coagulation proteins in frozen plasma" Blood Coag. Fibrinolysis, 12, 229-236, 2001*
(10) *CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular haemostasis assys; approved guideline". Fifth edition, 28, 5, 2008*
(11) *Occupational Safety and Health Standards; Bloodborne pathogens (29CFR1910.1030) Federal Register July 1, (1998) ; 6, p.267-280*
(12) *Directive du conseil de l'Europe (90/679/CEE) J. O. de la communauté européenne n°L374 du 31.12.1990, p.1-12*

| = Significant modifications

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			→	H2O	

Manufactured by
BIOLABO S.A.S.
Les Hautes Rives
02160 Maizy, France

Made in France

Distributed by
Kommanditgesellschaft Behnk Elektronik GmbH & Co.
Hans-Böckler-Ring 27
22851 Norderstedt, Germany

T. +49 (0)40-529 861 0
F. +49 (0)40-529 861 99
info@behnk.de

Latest revision: www.behnk.de