BIOLABO REAGENTS
www.biolabo.fr
MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives,
02160, Maizy, France

Factor IX Deficient Plasma

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

REF 13309 **R1** 6 x 1 mL

IVD

Made In France

I: corresponds to significant modifications

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
Fax: (33) 03 23 256 256
support@biolabo.fr
Latest revision: www.biolabo.fr

INTENDED USE

I This reagent is designated for professional use in laboratory (automated or semi-automated method). It allows the quantitative determination of Factor IX activity in citrated human plasma.
This test is realized with reagents BIOLABO as follows:

REF 13660 and 13670: BIO-SIL (APTT Silica)
REF 13560 and 13570: BIO-CK (APTT Kaolin)
REF 13565: $CaCl_2$ Solution 0.025 M
REF 13883: Owren Köller buffer dilute reference, control and patient's plasmas.

GENERALITIES (2) (3) (4) (7) (8)

Factor IX is a glycoprotein synthetized by the liver. The synthesis of biologically active F.IX (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 2 different ways:
- in the presence of Ca^{2+}, factor Xa activates F.IX to F. IXa
- Tissue factor/F. VIIa complex activates either F.IX or F.X
F. IXa forms an enzymatic complex with phospholipids, Ca^{2+} and F. VIIIa ; This complex then activates factor X to factor Xa

Decrease of F.IX activity is associated with:

- Hemophilia B (severity of hemophilia depends on F. IX: C level):
  - < 1% Severe hemophilia
  - 1 to 5% Moderate hemophilia
  - 5 to 25% mild hemophilia
- Hypovitaminosis K
  - AVK Treatment
  - Nutritional intake deficiency, disorders in absorption or metabolism of vitamin K (hemorrhagic disease of the newborn, cholestasis, treatment with antibiotics
- Liver failures
  - Cirrhosis
  - Hepatitis
- Decrease of the level of F. IX in the presence of F.IX inhibitor.

PRINCIPLE (1)

The assay consists in the measurement of the clotting time, in the presence of cephalin and activator, of a system in which all the factors are present and in excess except of factor IX which is derived from the sample being tested.

REAGENTS

R1 **F-IX** Deficient Plasma

Human Origin

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

SAFETY CAUTIONS

- Material Safety Data Sheet is available upon request.
- 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.

I 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.

REAGENTS PREPARATION

- Open the vial carefully and add exactly 1 mL of demineralized water**.**
- Recap and let stand for 15 minutes at room temperature.
- Before use, gently agitate to avoid the formation of foam.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, when stored and used as described, reagents are stable:

Unopened:
- Until expiry date stated on the label of the kit.

Once opened:
- R1 must be reconstituted immediately,

I Once reconstituted: 8 hours at 2-25°C.

SPECIMEN COLLECTION AND HANDLING (9) (10)

Citrated Plasma: Mix freshly drawn blood (9 Volumes) with buffered tri-sodium citrate solution 3.2% (1 volume).
Centrifuge for 10 min at 3000 g and extract supernatant.

Storage in plastic tube: 4h at 2-25°C

If quickly frozen: 15 days at -20°C, 1 month at -80°C (Thaw frozen plasmas at 37°C until complete thawing).

LIMITES (5) (6)

Heparins and Thrombin inhibitors (i.e. hirudin, argatroban …) present in the specimen to be tested may lead to under-estimation of the factor IX activity in the specimen.

The presence of Lupus anticoagulants may lead to an under-estimation 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

1. Basic medical analysis laboratory equipment.
2. Automated or semi-automated coagulation analyzer

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

CALIBRATION

- REF 13970: BIO CAL, reference plasma for calibration of coagulation tests

Calibration frequency depends on proper instrument functions and on the preservation of reagent.

QUALITY CONTROL

- REF 13971: COATROL1 Level 1
- REF 13972: COATROL 2 Level 2
- External quality control program.

It is recommended to control in the following cases:

- At least once a run
- At least once within 24 hours
- When changing vial of reagent
- After maintenance operations on the instrument

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVALS (2)

Plasma (adult)	Usually 60-150%

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

PERFORMANCES CHARACTERISTICS

On automatic analyzer SOLEA 100 at 37°C:

Precision:

Intra-Assay N = 20	level 1	level 2
Mean %	158	56
S.D. %	8.2	2.8
C.V. %	5.4	5.0

Inter-Assay N = 20	level 1	level 2
Mean %	132	47
S.D. %	9.4	3.3
C.V. %	7.1	7.0

Detection limit: 6 % of Factor IX

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

Interferences APTT Silica (sec):

Turbidity	No interference up to 0.404 abs
Bilirubin	Positive interference from 133 µmol/L
Hemoglobin	No interference up to 261 µmol/L

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

Onboard stability: Deficient plasma is stable 4 hours

Calibration Stability: Re-calibrate each day

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

PROCEDURE

Manual procedure on semi-automate BIO SOLEA 2, BIO SOLEA 4:

Prepare dilutions 1/10, 1/20, 1/40, 1/80 of REF 13970 BIO-CAL Reference Plasma in Owren Köller buffer

Pre-incubate PT Reagent at least 15 min at 37°C and mix gently.

Measure and record the clotting time for each dilution as follows:

Reference plasma diluted 1/10 to 1/80	0,1 mL
Deficient plasma	0,1 mL
APTT Reagent:	0,1mL
Incubate 3 minutes at 37°C.	
$CaCl_2$ 0,025 M:	0,1mL
The automatic countdown timer will start immediately after $CaCl_2$ addition and stop when the clot is formed.	

Do the same for controls and specimens to be tested (pre-diluted 1/10 in Owren Köller buffer):

Controls and specimens (diluted 1/10)	0,1 mL
Deficient Plasma	0,1 mL
APTT Reagent:	0,1mL
Incubate 3 minutes at 37°C.	
$CaCl_2$ 0,025 M:	0,1mL
The automatic countdown timer will start immediately after $CaCl_2$ addition and stop when the clot is formed.	

Automated procedure: Full detailed application available on request

- Performances and stability data have been validated on SOLEA 100 and 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 are available on request.

CALCULATION

Manual procedure:

Plot a Standard Curve using results obtained with dilutions 1/10 to 1/80 of reference plasma

Concentration % = f (Clotting Time)

Read the concentration of controls and samples reporting clotting time on the graph.

Automated and semi-automated procedure:

Patients results (seconds) will be automatically converted in % of Deficient Factor according to calibration curve.

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