BIOLABO
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BIO-SIL APTT Silica

Reagent for the determination of activated partial thromboplastin time (APTT) in human plasmas

REF 13660	**R1**	6 x 3 mL
REF 13670	**R1**	6 x 10 mL

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

CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

Reagent for chronometric determination of activated partial thromboplastin time (APTT) in human plasmas to investigate intrinsic coagulation pathway except platelets and to monitor heparin therapy. Laboratory professional use (manual or automated method).

GENERALITIES (1)

BIO-SIL Reagent is a basic coagulation screening test, useful to investigate the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II and I) except the platelets.

The main application of the APTT is to monitor heparin therapy.

APTT is also used to detect congenital and acquired deficiencies related to the factors mentioned above.

Prolonged APTT may require further investigations related to congenital or acquired deficiencies.

PRINCIPLE (4)

The BIO-SIL reagent contains a standardised amount of cephalin (platelet substitute) and a micronized activator (Silica). The reactional mixture in contact with CaCl2 solution 0,025M involves the recalcification of the plasma and the start of clotting reaction. The use of Silica avoids sedimentation of the reagent (standardised activation of XII factor).

REAGENTS (3)

R1 **BIO-SIL** Freeze dried Reagent

Cephalin (Rabbit cerebral tissues)

Silica

Before reconstitution:
Warning: Skin Sens.1: H317-May cause an allergic skin reaction,

P261: Avoid breathing dust, P302+P352: If on skin, wash with plenty of water, P333+P313 : If skin irritation or rash occurs, get medical advice P501: Dispose of contents/container in accordance with dangerous goods regulations. Classification due to: Silica < 1%. For more details refer to current Material Safety Data Sheet (MSDS)

Once reconstituted, working reagent is not classified as dangerous

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. 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.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment
2. Automated or semi-automated coagulation analyzer
3. Demineralised water for preparation of reagent
4. REF 13565: Calcium chloride 0.025 M

I REAGENTS PREPARATION

Vial R1:
Reconstitute immediately with the volume of demineralized water indicated
Mix gently until complete dissolution

STABILITY AND STORAGE

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

Unopened:

- Until expiry date stated on the label of the kit

Once opened:

- Reconstitute immediately the contents of vial R1.
- Transfer requested quantity, well recap vials and store at 2-8°C
- Working reagent is stable for 30 days at 2-8°C

Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (1) (7)

- Careful venepuncture:
- Blood/anticoagulant ratio: 4.5 mL of blood for 0.5 mL of trisodium citrate 2 H_2O 0.109 M.
- Avoid blood drawing with a syringe that could result in the formation of micro-clots.
- Centrifuge for 10 minutes at 2500 g as soon as possible and run the assay within 3 hours following the blood collection.
- Use plastic disposable test tubes to store plasmas refrigerated before testing.
- Patient's specimen on heparin therapy: centrifuge and run the assay within 1 hour following the blood collection.
- Simultaneously prepare a pool of at least 6 freshly drawn normal plasmas as reference.

I LIMITS (1) 2) (4) (5)

- Traumatic venepuncture may contaminate specimen with tissue thromboplastin and shorten APTT
- A difficult draw may also interfere with PTT used for heparin monitoring by neutralizing the heparin effect in sample due to release of Platelets Factor 4 (PF4).
- Heparin, depending on its origin and composition (calcium or sodium salt) has a different influence on the sensitivity of reagent.
- Mishrahi and al. indicate an easy procedure to determine the sensitivity of the method used in each laboratory and to inform the clinician in order to optimize the posology.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

CALIBRATION (4)

Results are method dependent.
The validity of the result depends on the time counting, the respect of reagent/specimen ratio and temperature.
Reference plasma time: Use normal human plasmas collected from healthy individual, either men or women aged from 18 to 55, not taking any medication and giving blood voluntarily.

QUALITY CONTROL

REF 13961	Control Plasmas Level 1	6 X 1 mL
REF 13962	Control Plasmas Level 2	6 X 1 mL
REF 13963	Control Plasmas Level 3	6 X 1 mL

Or

REF 13971	Coatrol 1	6 x 1 mL
REF 13972	Coatrol 2	6 x 1 mL

- 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.

I PERFORMANCES

On automatic analyser Thrombolyzer Compact X at 37°C.
Precision:

Within run N = 20	Level 1	Level 2	Level 3
Mean (sec.)	36.3	66.6	70.5
S.D. (sec.)	0.33	1.49	0.59
C.V. %	0.9	2.2	0.8

Between run N = 20	Level 1	Level 2	Level 3
Mean (sec.)	42.2	63.4	69.1
S.D. (sec.)	0.99	1.19	0.77
C.V. %	2.3	1.9	1.1

On automatic analyser SOLEA 100 at 37°C:
Precision:

Within run N = 20	Level 1	Level 2	Level 3
Mean (sec.)	36	66	73
S.D. (sec.)	0.48	0.88	0.58
C.V. %	1.3	1.3	0.8

Between run N = 20	Level 1	Level 2	Level 3
Mean (sec.)	41	62	67
S.D. (sec.)	0.84	1.14	1.64
C.V. %	2.1	1.9	2.4

Comparison with commercially available reagent:

202 plasmas located between 22 and 69 sec were tested:

y = 1.6631 x – 18.053 r= 0.9077

Interferences on APTT (sec):

Turbidity	No interference up to 0,543 abs
Total 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: 30 days when kept 8 hours per days onboard (15-25°C) and remaining time at 2-8°C

REFERENCE INTERVALS (1) (5) (6)

Reference range varies with reagent-instrument combinations and with local conditions (population…).

In general: lower limit of normal 20-25 sec, upper limit < 35 sec

Therefore, each laboratory should determine its reference intervals on a panel of normal plasmas.

In this case, generally, a value is considered as normal if within mean value +/- 2 standards deviations (+/-2SD).

For example, 55 normal plasmas have been tested on SOLEA 100:

The observed mean is 29,7 sec and standard deviation 2,3 sec

The APTT is normally prolonged in new-borns/infant.

The APTT gradually decreases into adult range by 6 months

PROCEDURE

Manual method on semi-automate BIO SOLEA2, BIO SOLEA4

Pre-warm Calcium chloride 0.025 M REF 13565 at 37°C

Well homogenised Reagent R1	0.1 mL
Specimen (Controls, Reference, Patients)	0.1 mL
Mix, incubate exactly for 3 minutes at 37°C.	
REF 13565 : $CaCl_2$ 0.025 M	0.1 mL
The automatic Countdown timer will start immediately after $CaCl_2$ addition and stop when the clot is formed.	

Automated Procedure:

Full detailed applications available on request.

1. Performances and stability data have been validated on SOLEA100 and Thrombolyzer Compact X.
2. With manual procedure performances and stability data must be validated by user.

CALCULATION (5)

Results may be expressed as follows:

- In seconds (Patient time and Reference plasma time)
- In ratio (Patient time/Reference plasma time)

Note: Reference plasma time: see § Calibration

REFERENCES

(1) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.46-47*
(2) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-447 à 3-448*
(3) *Bell W.N., Alton H.G., Nature, 1954, **174**, 880-881.*
(4) *Struver G.P., Bittner D.L. Am. J. Clin. Path. 1962, **38**, 473-481).*
(5) *Misrahi N., Manet L., Conard J., Samama M., Act. Pharm. Biol. Clin. 1981, **1**, 81-85.*
(6) *LEVIN HILLMAN C.R., LUSHER J.M.: "Determining the sensitivity of coagulation screening reagents: a simplified method". Lab. Med., 13, 3, 162-165, 1982*
(7) *Etude des différents paramètres intervenant dans les variables préanalytiques (revue de littérature) ». Sang Thromb. Vaiss., **10**, p.5-18 (1998)*

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