BIOLABO
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BIO-TP Prothrombin Time (PT)

For determination of Prothrombin Time (%) and INR in human plasma

REF	13885	**R1**	10 x 2 mL	**R2**	1 x 25 mL
REF	13880	**R1**	6 x 4 mL	**R2**	1 x 25 mL
REF	13881	**R1**	6 x 12 mL	**R2**	1 x 80 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (semi-automated or automated method).
It allows the quantitative determination of Prothrombin Time (%) and INR (International normalized ratio) in human plasma to screen the extrinsic coagulation pathway and monitor AVK treatment.

GENERALITIES (1) (6) (7)

The Prothrombin time (PT) is a useful basic coagulation screening test to investigate the extrinsic coagulation pathway.
PT (in sec.) converted into PT (%) allows the evaluation of the prothrombinic activity, referring to a normal plasma (100 %).
A deficient prothrombinic activity is found in the following clinical states:

- Hemorrhagic disease of the newborn.
- Liver failure (cirrhosis, hepatitis...).
- Vitamin K deficiency or treatment with vitamin K antagonists.
- Congenital deficits in one of the factors associated with the prothrombinic complex, real prothrombin (factor II), proaccelerin (factor V), proconvertin (factor VII) and Stuart's factor (factor X)
- Circulating anticoagulants
- Fibrinolysis
- DIC (disseminated intravascular coagulation).

Monitoring of treatment with vitamin K antagonists:
The PT (in sec.) may be converted into INR (International Normalised Ratio). In that case, the origin of the thromboplastin has no incidence on the determination of the expected values. An international standardisation about INR reference intervals has been established for treatment and prophylaxis of venous and arterial thromboembolisms.
Avoid results in INR in the case of pre-operative check-up or investigations for liver diseases.

PRINCIPLE (4)

Quick and al. method. Principle as follows:
The clotting time is measured at 37°C in the presence of tissular thromboplastin and calcium. It reflects the activity of Factor II (prothrombin),V (proaccelerin), VII (proconvertin), X (Stuart Factor) and fibrinogen. The measured time is converted into PT (%) or INR

REAGENTS

R1 **BIO-TP** Freeze-dried Thromboplastin

Rabbit cerebral tissue

R2 **BIO-TP** Reconstitution Buffer

HEPES Buffer, Stabilizer

According to 1272/2008 regulation, these reagents are 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.

I REAGENTS PREPARATION

Add promptly to the contents of the vial R1 the amount of reconstitution buffer (vial R2) stated on the label.
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 reconstituted:

- Transfer requested quantity, well recap vials and store at 2-8°C
- Working Reagent is stable for:
  - ✓ 8h00 at room temperature
  - ✓ 5 days at 2-8°C.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (2) (8)

Careful venipuncture.

- Blood/anticoagulant ratio: 4.5 mL of blood for 0.5 mL of sodium 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.
- Run the assay within 4 hours after collection, storing plasma at room temperature (15-25°C).
- Collection on citrate Hepes tube increases the specimen stability up to 8 hours.

LIMITS (2) (3)

Contamination by Thromboplastin or hemolysed specimens may also shorten the result of PT (in sec.).
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 coagulation analyzer SOLEA 100
3. Semi-automated coagulation analyzer BIO SOLEA 2, BIO SOLEA 4
4. REF 13883 Owren Köller Buffer (Thivolle Line): manual procedure only

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 13965: TP CALSET (INR, PT%)
- Or use enclosed Calculation Paper Board

Automated method on SOLEA 100:
Perform a calibration using REF 13965 as indicated in the specific application

On semi-automate BIO SOLEA 2, BIO SOLEA 4:

Enter the mean of the clotting time for each level of REF 13965, and the corresponding % and INR.

Manual method:
Use Calculation Paper Board, or :
Calculate INR results using MNPT and ISI (see § Calculation)
Calculate % results using normal plasmas pool dilutions in REF 13883 and plot Thivolle line.

- ISI (International Sensitivity Index): See enclosed Paper Board
  It has been determined by testing human plasmas with this Thromboplastin and with an Internal Reference Thromboplastin traceable to RBT16 (WHO International Standard Thromboplastin, Rabbit plain).
  Obtained PT (sec values) with the 2 thromboplastins were plotted on log to log graph and the slope was calculated. ISI was then calculated multiplying the slope by ISI of the Internal Reference Thromboplastin.
- MNPT (Mean normal plasma time):
  Prepare a pool of freshly collected normal plasmas. Measure in triplicate the clotting time and calculate the mean.

I PERFORMANCES

On automatic analyzer Thrombolyzer Compact X (precision):

Within run N = 20	Level 1	Level 2	Level 3
Mean (%)	91.3	36.6	21.4
S.D. (%)	0.96	0.60	0.49
C.V. %	1.1	1.6	2.3

Between run N = 20	Level 1	Level 2	Level 3
Mean (%)	91.1	35.6	20.3
S.D. (%)	1.85	1.12	0.61
C.V. %	2.0	3.2	3.0

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

Within run N = 20	Level 1	Level 2	Level 3
Mean %	89	36	22
S.D. (%)	1,3	0,57	0,40
C.V. %	1,46	1,57	1,85

Between run N = 20	Level 1	Level 2	Level 3
Mean %	89	35	21
S.D. (%)	1,66	1,47	0,64
C.V. %:	1,86	4,17	3,08

Comparison with commercially available reagent (same method):

170 plasmas located between 14% and 110% were tested:

y = 1,0287 x + 0,1601 r= 0,9863

Interferences on PT (sec, INR):

Turbidity	No interference up to 0,390 abs
Low Molecular weight heparin	Positive interference from 0.11 IU anti Xa
Bilirubin	Positive interference from 171 µmol/L
Hemoglobin	No interference up to 258 µmol/L

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

Onboard stability: at least 24 hours when kept 8 hours per days onboard

Calibration Stability: 6 weeks

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

REFERENCE INTERVALS (2) (6) (9)

Prothrombin Time (sec):
Usually between 11 and 16 seconds (depending on the reagent).

PT (%):
✓Ranging between 70 to 100 %.
✓Values over 100 % are considered as normal.

INR: Oral anticoagulant therapy (OAT):

Indications	Therapeutic range in INR		PT (%) Rabbit thromboplastin
	Target	Acceptable range	
Pre-operative and during surgery: *Hip surgery	2.5	2.0 – 3.0	35 %
*Other surgery	2.0	1.5 – 2.5	40 %
Venous thrombosis prophylaxis	2.5	2.0 – 3.0	35 %
Evolutive phlebitis, pulmonary embolism, recurrent phlebitis	3.0	2.0 – 4.0	27 %
Arterial prophylaxis, mechanical prosthetic valves	3.5	3.0 – 4.5	25 %

QUALITY CONTROL

REF 13961	Control Plasma level 1	6 x 1 mL
REF 13962	Control Plasma level 2	6 x 1 mL
REF 13963	Control Plasma 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.

PROCEDURE

Manual procedure on semi-automate BIO SOLEA 2, BIO SOLEA 4:
Pre-incubate Working Reagent at least 15 min at 37°C and mix gently.
Refer to §Calibration

Plasma	0.1 mL
Incubate for 2 minutes at 37°C	
Working reagent (homogenized)	0.2 mL
The automatic countdown timer will start immediately after working reagent 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 (6)

$$\text{INR (result)} = \left(\frac{\text{Patient's time}}{\text{MNPT}} \right)^{\text{ISI}}$$

Manual Procedure:

- Use enclosed Paper Board:
  ✓Select the column corresponding to "MNPT".
  ✓Identify the patient's PT (sec) in this column.
  ✓On the same line, refer to the corresponding PT (%) or INR.

Or

- Use the Thivolle line (see § Calibration)

Semi-automated procedure and automated procedure:
Results (INR, %) will be calculated automatically after uploading parameters in the system using calibration curve.

REFERENCES

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