CE
In Vitro
Diagnostic

Behnk Elektronik

BE PT LI Thromboplastin low ISI

Reagent for determination of Prothrombin time (PT) in human plasma

REF 771100: RE (5 x 5 mL), DIL (2 x 15 mL)
REF 771101: RE (8 x 12 mL), DIL (8 x 12 mL)

PRINCIPLE [4]

Quick method: tissue thromboplastin and calcium are added to citrated plasma, activating the factors of extrinsic coagulation pathway. The clotting time (time to formation of a fibrin clot) is measured at 37 °C.

CLINICAL SIGNIFICANCE [1] [6] [7]

The Prothrombin Time is used as a screening test to detect disorders in the extrinsic coagulation pathway.
The results in seconds are converted into percentages to evaluate the prothrombinic activity in comparison to a reference plasma consider as 100%.
A deficient prothrombinic activity can occur in the following cases:
Hepatic disease; Hemorrhagic disease of new born; Congenital factor deficiency (factors II, V, VII or X); Vitamin K deficiency; Vitamin K antagonist treatment; Circulating anticoagulants; Fibrinolysis; Disseminated intravascular coagulation (DIC)
The results in seconds are converted into INR (International Normalized Ratio) in the particular use of monitoring oral anticoagulant therapy (Vitamin K antagonists).

REAGENTS

RE **PT LI** Reagent
Thromboplastin (lyophilized from rabbit brain).
According to 1272/2008 regulation, this reagent is not classified as dangerous.

DIL **PT Diluent** Diluent **Attention**
Hepes buffer, calcium.

Skin Sens.1: H317 - May cause an allergic skin reaction
P261: avoid breathing sprays, P280: Wear protective gloves/protective clothing/eye protection/face protection, P302+352: IF ON SKIN: Wash with soap and water.
P333+313: If skin irritation or a rash occurs: Get medical advice/attention,
P501: Dispose of contents/container in accordance with dangerous goods regulations
Classification due to: Nickel Sulfate < 1%. For more details refer to current Material Safety Data Sheet (MSDS)
Once reconstituted: Working Reagent (vial RE) is classified as Buffer (vial DIL)

SAFETY CAUTIONS

Behnk reagent kits are designated for professional in vitro diagnostic use.
Good Laboratory Practices must be applied during use of reagents, reference or control plasmas, and human samples and should be handled as potentially infectious.
For further information, Material Safety datasheet is available upon request.
Dispose of waste in accordance with the local regulations.

PREPARATION OF REAGENTS

RE: Reconstitute the lyophilisate with the amount of DIL indicated on the label of RE. Cap vials and mix gently the RE vial until complete dissolution.
DIL: Ready for use

STABILITY AND STORAGE

Unopened vials stored at 2-8 °C are stable until the expiry date stated on the label.
RE: after reconstitution the working reagent is stable 5 days at 2-8 °C, or 6 h at 37 °C.
DIL: once opened, if stored at 2-8 °C and free from contamination, contents of the vial is stable until the expiry date stated on the label.
Do not use any reagent after expiry date.

SAMPLES COLLECTION AND HANDLING [2] [8]

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (trisodium 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 10 minutes at 2500 g.
The specimen is stable 4 hours after collection, at room temperature (15-25 °C).
Collection on Citrate Hepes tube increases the sample stability to 8 hours.

LIMITS [2] [3]

Samples contaminated by thromboplastin or hemolysis may shorten the clotting time.
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
REF 050813: Magnetic stirrers 8 x 1.5 mm, for Behnk Thrombolyzer series.

EXPECTED VALUES [2] [6]

PT (sec): Normal values between 11 and 16 sec.
PT (%): Normal values 70 % to 100 %. Values over 100 % have no clinical significance.
PT (INR): It is advised to each laboratory to establish its own reference range of expected values.

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 reagent 15 min to reach a temperature of 37 °C and mix gently before use:

- Plasma: 100 µL

Incubate for 120 sec at 37 °C

- Thromboplastin (37 °C): 200 µL

The automatic countdown timer will start immediately after Thromboplastin 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.

CALIBRATION

PT INR and % with Calibrator Set
Use calibrator set REF 775200: BE Cal Set traceable to RBT16 (WHO International Standard Thromboplastin, Rabbit plain).

- Automated method on Behnk Thrombolyzer series**:** Perform a Calibrator Set calibration with BE Cal Set.
- Manual method on semi-automated systems (PT %): Prepare a calibration curve with Cal1, Cal2 and Cal3. Measure in triplicate the clotting time of each level.

PT INR with MNPT and ISI (all methods)

- MNPT (Mean Normal Prothrombin Time)

To determine the MNPT prepare a pool of freshly collected normal plasmas. Measure in triplicate the clotting time and calculate the mean.

- ISI (International Sensitivity Index)**:** Refer to the batch specific table.

CALCULATION [6]

PT INR and PT % with Calibrator Set

- Automated method on Behnk Thrombolyzer series**:** PT INR and PT % will be calculated automatically according to two calibration curves.
- Semi-automated systems: Enter the mean of the clotting time found for each BE Cal Set plasma and the corresponding PT % in the system. PT % concentration will be calculated automatically according to calibration curve.

PT INR with MNPT and ISI (all methods)
The MNPT and ISI are to be used to calculate result in INR.

- Calculate INR as follows: $INR = (\text{Patient time} / \text{MNPT})^{ISI}$
- Refer to enclosed batch specific table, selecting the suitable column according the MNPT. Identify the raw corresponding to patient's result and refer to the result in PT % and PT INR indicated in column "%" and "INR".
- For semi-automated systems and Behnk Thrombolyzer series the INR will be calculated automatically after input in the system.

PERFORMANCES

The within run and between run studies were performed with normal and abnormal plasma on Thrombolyzer Compact X.

Within run ***N = 20***	Normal Plasma	High Plasma
Mean (%)	96.6	30.3
S.D. (%)	0.98	0.54
C.V. %	1.01	1.77

Between run ***N = 20***	Normal Plasma	High Plasma
Mean (%)	96	30.4
S.D. (%)	1.81	0.99
C.V. %	1.88	3.26

Comparison with commercially available reagent, same method: 167 plasmas located between 14% and 110%:
y = 1.1376x – 1.4301 r= 0.9958
Interferences (sec, INR):

Turbidity	No interference up to 731 mg/dL of Triglycerides
Low Molecular weight heparin	Positive interference from 0.114 IU anti Xa
Unfractionated heparin	Positive interference from 0.038 IU anti Xa
Bilirubin	Positive interference from 228 µmol/L
Hemoglobin	No interference up to 240 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) *Caen J., Larrieu MJ, Samama M : « L'hémostase. Méthodes d'exploration et diagnostic pratique » Paris : L'Expension Scientfique, p.344-347, (1975).*
(2) *Clinical Guide to Laboratory Test, 3rd Ed., N.W. TIETZ (1995) p.526-529*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-513 à 3-517*
(4) *Quick A. J.- J. Am. Assoc., (1938), **110**,p. 1658-1662*
(5) *Duckert F., Marbet G.A. - Méd., et Hyg., (1977), **35**, p. 911*
(6) *Goguel A.F. - Feuillets de Biologie, (1985), **36**, (146) p. 25-28.*
(7) *Houbouyan-Reveillard et al. Spectra biologie (2003) vol.22, n°132 p.33-37*
(8) *Neofotistos D, Oropeza M., Ts'ao C-H : « Stability of plasma for add-on PT and PTT tests » Am. J. Clin. Pathol. 109, 6, 758-763, (1998).*

Manufacturer	Use by	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		Σ	→	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

Latest revision : www.behnk.de

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

Revision : 20171219