CE IVD

Precision in Hemostasis

Behnk Elektronik

BE PT LI Thromboplastin low ISI

Reagent for the determination of Prothrombin Time
in human citrated 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)

| INTENDED USE

Reagent (Rabbit brain Thromboplastin) for *in vitro* quantitative chronometric determination of prothrombin time (%) and INR values in human citrated plasma.
Suitable for use with manual procedure on semi automated coagulometer, or on automated coagulation analyzer with BE Cal Set Calibration Plasma PT.
Its intended use is the screening of the extrinsic coagulation pathway and the monitoring of oral vitamin K antagonist therapy using the INR.
This Device can also be used in conjunction with the relevant deficient plasmas, for the determination of the activity of coagulation Factors II, V, VII, X in human citrated plasma.
For clinical laboratory use by healthcare professionals.

PRINCIPLE [4]

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

GENERALITIES [1] [6] [7]

The Prothrombin time is a useful basic coagulation screening test to investigate the extrinsic coagulation pathway. PT (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, Factors II, V, VII and X.
- Circulating anticoagulants.
- Fibrinolysis.
- DIC (Disseminated Intravascular Coagulation).

Monitoring of treatment with vitamin K antagonists:
The PT (sec) may be converted into INR. In that case, the origin of the thromboplastin has no incidence on the determination of the expected values. An international standardization 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.

| REAGENTS

RE	**PT LI**	Freeze-dried Thromboplastin
Rabbit cerebral tissue		1.1%
Non active Excipient		98.9%

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

DIL	**PT Diluent**	Reconstitution Buffer	**Attention**
Pipes Buffer		pH 6.8 (25°C)	
Calcium		0.4%	

According to (EC) 1272/2008 regulation, DIL is classified:
GSH07: 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 Safety Data Sheet (SDS)
Once reconstituted: Working Reagent (vial RE) is classified as Buffer (vial DIL)

SAFETY CAUTIONS

- Refer to current Safety Data Sheet (SDS) available on request or on www.behnk.de.
- 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.

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 away from light at 2-8 °C are stable until the expiry date stated on the label.
RE: Stability after reconstitution:

- 2-8 °C 7 days
- On board Stability (OBS)* 2 days
- Laboratory mode** 5 days (INR), 2 days (%)

*16-22 °C, stirred.
**Laboratory mode = 8 hours on board; 16 hours well capped in the original vial at 2-8°C.

| SAMPLES COLLECTION AND HANDLING [2] [8]

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (sodium citrate solution 0.109 M).
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 24 hours at room temperature (15-25°C).

LIMITS [2] [3]

Samples contaminated by thromboplastin or hemolysis may shorten the result of PT (sec).
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.

REFERENCE RANGE [2] [6] [9]

PT (sec): Normal values 11 to 16 sec.
PT (%): Normal values 70 % to 100 %. Values over 100 % are considered as normal.

PT (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 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). These data are valid for all instruments in Thrombolyzer series.
- With manual procedure and on other automated coagulation analyzer, performances and stability data must be validated by user.

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CALIBRATION [10] [11]

PT INR and % with Calibrator Set:
Use calibrator set REF 775200: BE Cal Set
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): See enclosed batch specific table.

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.

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 must be used to calculate result in INR.
Calculate INR as follows: $INR = (Patient's\ time / MNPT)^{ISI}$

Manual Procedure:
Refer to enclosed batch specific table, selecting the suitable column according to the MNPT:

- Identify the patient's PT (sec) in this column.
- On the same line, refer to the corresponding PT (%) or INR.

For semi-automated systems and Behnk Thrombolyzer series, the INR will be calculated automatically after input in the system.

PERFORMANCES

On the automated Thrombolyzer Compact X analyzer:

Precision: Repeatability (Within run) and Reproducibility (Between run):

Within run N = 20	Level 1	Level 2	Level 3
Mean (%)	96.6	30.3	16.4
S.D. (%)	0.98	0.54	0.39
C.V. (%)	1.0	1.8	2.4

Between run N = 20	Level 1	Level 2	Level 3
Mean (%)	96	30.4	16.7
S.D. (%)	1.81	0.99	0.47
C.V. (%)	1.9	3.3	2.8

Comparison with commercially available reagent, same method: 167 plasmas located between 14% and 110%:
y = 1.1376 x – 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
Total Bilirubin	Positive interference from 228 µmol/L
Hemoglobin	No interference up to 258 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

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(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.928-929.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-513 to 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) *CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline". Fifth Edition, **28**, 5, 2008*
(9) *Sampol J., Arnoux D., Boutière B. : « Manuel d'hémostase » Paris, Ed. Elsevier, 147-163 (1995).*
(10) *RBT16 certificate (WHO)*
(11) *BEESER H.: "Critical evaluation of the so far experience using the WHO model of prothrombin time calibration and outlook for further development". Haemostasis, 18, suppl., 2, 181-182, 1988*

| = Significant modifications

IFU_771100-771101-EN_V04_20260525

Table of symbols

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		Σ	→	H_2O	

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