

PT-Phen™ LRT**REF** CK584K-RUO, **R** 6 vials x 5 mL**REF** CK584L-RUO, **R** 12 vials x 5 mL**REF** CK586K-RUO, **R** 6 vials x 20 mL**REF** CK586L-RUO, **R** 12 vials x 20 mL**FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.**

English, revision: 10-2024

INTENDED USE:

Clotting method for the *in vitro* quantitative determination of Prothrombin Time (PT), in human citrated plasma, using an automated method. This device of *in vitro* use is intended for professional use in the laboratory. **This kit is for research use only and must not be used for patient diagnosis or treatment.**

SUMMARY AND EXPLANATION:**Technical:¹**

PT is a one-stage test based on the Quick method. PT assesses the overall activity of clotting factors of the extrinsic pathway (coagulation triggered by contact with thromboplastin expressed by specific cells).

PT does not explore factor deficiencies of the intrinsic coagulation pathway (Factors VIII, IX, XI, and XII), platelets, Factor XIII or natural inhibitors of coagulation (Antithrombin, Protein C and Protein S).

PRINCIPLE:

PT is the determination of a clotting time at 37°C in the presence of thromboplastin and calcium, by initiation of activation of the extrinsic coagulation pathway (complex TF-FVIIa activated factor X, then generation of thrombin, leading to fibrin formation). The clotting time depends on the activity of extrinsic pathway coagulation factors (II, V, VII, X, Fib). Measured PT can be converted to concentration (PT%), or expressed as a ratio (PT ratio), or as recommended "International Normalized Ratio (INR)".

The reagent includes a heparin neutralizing substance.

REAGENTS:

R **PT-Phen LRT reagent**, at approximately 200 ng/mL of recombinant human Tissue Factor (rec(h)TF), liquid form. Contains BSA, a specific heparin neutralizing substance, preservatives and stabilizers.

The product is classified as non-hazardous and is not subject to labeling according to EC Regulation No. 1272/2008 [CLP]

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.

REAGENT PREPARATION:

R Reagent is ready to use; mix vigorously while avoiding formation of foam and load it directly on the analyzer following Application Guide instruction.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **1 month at 2-8°C.**
- **Analyzer on-board stability: see the specific Application Guide.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Specific calibrators and controls:

Product Name	Reference
EASYPLASMA™ Control Set	225601-RUO
BIOPHEN™ Normal Control Plasma	223201-RUO
BIOPHEN™ Abnormal Control Plasma	223301-RUO
PT Calibrator Set	SC084K-RUO

- **Normal Individual Plasmas for MNPT (Mean Normal Prothrombin Time) assessment¹**
- **Frozen normal Plasma Pool for internal control**
- Automatic analyzer for clotting assays such as: CS-series, CN-series.
- Laboratory material.

Please note that the applications on other analyzers can be validated by the instrument manufacturer under their responsibility as long as the intended purpose is not modified.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.

Samples should be collected, prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5⁴ guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references^{4,5}.

PROCEDURE:

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and performance information and supersede the information in these Instructions for Use.

TRACEABILITY:

The International Sensitivity Index (ISI) value assignment of each PT-Phen™ LRT lot is determined against an in house standard, related to the corresponding WHO International Standard and using VKA samples¹. Certificate of traceability is available upon request.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.

For PT% assessment using SC084K:

Include the quality controls with each series, as per good laboratory practice, in order to validate the test.

A new calibration curve should be established:

- preferably for each test series,
- for each new reagent batch,
- after analyzer maintenance,
- when the measured quality control values fall outside the acceptance range for the method.

Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

For INR assessment using ISI value provide into the flyer:

Include the quality controls with each series as well as frozen normal plasma pool regularly (e.g.: every 3 months), to validate the test.

New MNPT assessment should be established:

- for each new reagent batch³
- after significant analyzer maintenance³
- when the measured quality control values fall outside the acceptance range for the method³
- when PT seconds (PT (sec)) of a same normal plasma pool used is measured +/- 1.0 second from initial measurement (internal control)

Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- The prothrombin time obtained (PT sec) may be converted to percent of normal value (PT%), expressed as a ratio (PT ratio), or as "International Normalized Ratio (INR)".
- The principles are as follows^{1,2,3}:

INR equation

The INR value is determined by calculation method. This method depends on the ISI and MNPT values using following equation^{1,3}:

$$INR = \left[\frac{PT(sec) \text{ plasma}}{MNPT} \right]^{ISI}$$

With MNPT = Mean Normal Prothrombin Time (geometric mean from at least 20 plasmas from individual healthy adult donors, as per current local recommendations or guidelines¹)

And ISI = International Sensitivity Index, for the combination of reagent lot/method or instrument used, determined by reference to WHO reference recombinant human thromboplastin.

The ISI for the reagent lot used is indicated on the flyer provided with the kit. MNPT has to be established by end-user. MNPT established by the manufacturer and provided into the flyer could be used after internal validation on the own end user responsibility.

Prothrombin time ratio

PT Ratio = (PT (sec) plasma) / (MNPT), with MNPT established by end-user. Same measure to be considered as above for MNPT management.

Direct determination of PT% value^{3,4}

Realize a PT% calibration curve by determining the clotting times of PT Calibrator Set (SC084K) and plotting log-log on abscissae the PT% values and on ordinates the corresponding clotting times (seconds). The PT% value of tested plasma is directly obtained on the calibration curve.

This method is independent of International Sensitivity Index (ISI) and MNPT values.

The results obtained should be for research use only and must not be used for patient diagnosis or treatment.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting no limp appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- User defined modifications are not supported by HYPHEN BioMed as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in HYPHEN BioMed Application Guides or these Instructions for Use.
- Mechanical or photo-optical systems can be used with PT-Phen™ LRT reagent, however the clot detection method may vary from one to another³, so it is recommended to not compare the PT (sec) results obtained with different detection methods. Also, results obtained with different thromboplastins, especially from different origins, may vary³.
- Many substances, anti-thrombin and anti-Xa inhibitors, and common variables may affect PT results^{1,3}; any unexpected result should be further explored. The reagent includes a heparin neutralizing substance and is insensitive to heparins up to 2 IU/mL (UFH/LMWH).
- Reagent is not sensitive to moderate Lupus anticoagulants (LA) and Factor V-L (tested on heterozygous patients). Inhibitors such as LA may prolong PT.³
- Many variables may affect clotting times (e.g. working conditions such as temperature, water quality, pH, system used, sampling, storage, population)¹.
- Each laboratory has to determine its own normal range in its specific test conditions.

PERFORMANCES:

Performances studies were conducted as described in CLSI guidelines. The following performance data represent typical results and are not to be regarded as specifications for PT-Phen™ LRT. Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.

Analytical performances

Measuring Range

		PT %
Limit of Blank (LOB)		0.0
Limit of Detection (LOD)		0.2
Measuring range	Direct	12-96
	With extrapolation	12-120

*Expected calculated value for INR from 1 to 10.

Precision

Samples	PT%					
	Repeatability				Within-Laboratory	
	n	Mean	SD	CV (%)	SD	CV (%)
Normal Control plasma	80	79.4	0.64	0.8	0.80	1.0
Anormal Control plasma	80	37.2	0.20	0.5	0.45	1.2
Control INR 3.25	80	16.6	0.04	0.2	0.38	2.3
Normal pool plasma	80	81.6	0.56	0.7	2.40	2.9
Samples	PT Sec					
	Repeatability				Within-Laboratory	
	n	Mean	SD	CV (%)	SD	CV (%)
Normal Control plasma	80	12.5	0.03	0.3	0.10	0.8
Anormal Control plasma	80	22.5	0.08	0.4	0.25	1.1
Control INR 3.25	80	42.6	0.12	0.3	0.70	1.6
Normal pool plasma	80	12.3	0.04	0.3	0.23	1.9

Interfering substances

No interferences up to:

Hemoglobin	1000 mg/dL	Triglycerides	219 mg/dL*
Bilirubin (Free)	15 mg/dL	Heparin (UFH/LMWH)	2 IU/mL
Bilirubin (Conjugated)	60 mg/dL	Dabigatran	50 ng/mL
Apixaban	50 ng/mL	Lupus	Not sensitive
V-L factor	Not sensitive		

*Value for CS series.

REFERENCES:

- Clinical and Laboratory Standards Institute (CLSI). One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; 3rd ed. CLSI Guideline H47- H47Ed3, 2023.
- Poller L. *et al.* The prothrombin time/international normalized ratio (PT/INR) Line: derivation of local INR with commercial thromboplastins and coagulometers – two independent studies. J. Thromb Haemost. 2011.
- Clinical and Laboratory Standards Institute; Procedures for validation of INR and Local Calibration of PT/INR Systems; H54-A, Vol.25, number 23.
- CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
- Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

Changes compared to the previous version.

REF	Catalog number	LOT	Batch code	RUO	Product for <i>in-vitro</i> research use, only
Rx	Numerical < x > identification of reagent	i	See instructions for use	MA	See instructions in Method Application guide
	Temperature limitation		Manufacturer		Use by YYYY-MM-DD
CONTENTS	Contents	→	Reconstitution volume	EXP	Expiration date
CONTAINS	Contains				