LIAPHEN™ Protein C

REF 120003-RUO

R1 4 vials x 5 mL, R2 4 vials x 2.3 mL

FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

Immuno-turbidimetric method for the *in vitro* quantitative determination of Protein C antigen in human citrated plasma, using a manual and automated method.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical: [1,2]
Protein C (PC) is a vitamin K dependent glycoprotein, which inhibits and regulates coagulation through specific cleavages of Factors Va and VIIIa, suppressing their procoagulant cofactor activity.

PRINCIPLE:

LIAPHEN™ Protein C is an immune-turbidimetric method, based on antigen-antibody reaction: Protein C antigen (PC:Ag) of the sample reacts with latex particles sensitized with rabbit antibodies, leading to latex particles agglutination. This agglutination can be directly detected through the change of absorbance. The absorbance change is directly proportional to the amount of PC:Ag in the sample.

REAGENTS:

R1 **Reaction Buffer**, liquid form. Contains Disodium dihydrogen ethylenediaminetetraacetate, BSA, preservatives and stabilizers.
R2 **Latex,** anti-Protein C antibodies-coated latex particles at approximately 0.25%, liquid form. Contains BSA, 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:

- This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
- Use only the reagents from the same batch of kits.
- Waste should be disposed of in accordance with applicable local regulations.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize by gentle inversion, 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.

R1 R2 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **3 months** at 2-8°C.
- **Do not freeze**
- **Stability on board of the analyzer: see the specific Application Guide.**

Combination of storage are not recommended.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Diluent: Imidazole Buffer (AR021B-RUO/AR021K-RUO/AR021L-RUO/AR021M-RUO/AR021N-RUO).
- Specific calibrator and controls with known PC:Ag titration, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101-RUO
BIOPHEN™ Normal Control Plasma	223201-RUO
BIOPHEN™ Abnormal Control Plasma	223301-RUO

- Automatic analyzer for immuno-turbidimetric assays such as: CS-series, CN-series.
- Laboratory material.

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-A5[3] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[3,4].

PROCEDURE:

Automated method:

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.

Assay method:

1. Reconstitute the reference preparation or plasma calibrator, and plasma controls as indicated on the specific instructions or according to internal practice.
Prepare the calibration concentrations from 0 to 150% PC:Ag (0-25-50-75-100-150 % PC:Ag in diluent), the **1:5** dilution corresponding to the indicated "C" concentration of PC:Ag for the commercial calibrator.

2. Program the specimens and controls dilution in diluent, as described in the table below:

Specimens	Reference	Dilution
Controls	223201-RUO/ 223301-RUO	**1:5**
Specimen	n.a	**1:5**

Establish the calibration curve and validate it with the quality controls. The diluted specimens should be tested extemporaneously if they are stored at room temperature (18-25°C). The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. As an example, the below table shows the scheme for CS-series application. Dispense the following to the reaction cuvettes incubated at **37°C** (directly managed by the analyzer):

	Volume
Calibrators, specimens or controls **diluted** in diluent	10 µL
Diluent	10 µL
R1 **Reaction Buffer**	165 µL
Incubate **at 37°C** for **150 sec.**	
R2 **Latex**	75 µL
Mix and measure the optical density continuously (**between 20 and 60 sec**) at **575nm,** at **37°C.**	

If a reaction volume other than that specified above is required for the method used, the respective volume ratios must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
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, and at least for each new reagent batch, or after analyzer maintenance, or 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.

RESULTS:

- For the manual endpoint method, plot the calibration curve lin-lin, with the OD 575 nm along the Y-axis and the PC:Ag concentration, expressed as %, along the X-axis.
- The concentration of PC:Ag (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- Lot to lot variability measured on 3 lots is: %CV ≤ 10%.

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 limpid 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.

PERFORMANCES:

Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.
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 LIAPHEN™ Protein C.

Analytical performances
Measuring Range

On CS-series	Limit of Blank (LOB)		0.2%
	Limit of Detection (LOD)		0.8%
	Limit of Quantification (LOQ)		2.9%
	Linearity	Direct	3 – 113%
		With re-dilution	3 – 142%

Precision (on CS-series)

Samples	Within-Laboratory		
	n	Mean (%)	CV (%)
Normal Control	80	76.6	2.6
Abnormal Control	80	31.2	3.6
Plasma pool	80	73.7	2.8

Interfering substances
No interferences, on CS-series, up to:

Bilirubin C	60 mg/dL	Dabigatran	500 ng/mL
Bilirubin F	60 mg/dL	Apixaban	500 ng/mL
Intralipids	1000 mg/dL	Edoxaban	500 ng/mL
Hemoglobin	1000 mg/dL	Rivaroxaban	500 ng/mL
UFH	5 IU/mL	Rheumatoid factors	500 IU/mL
LMWH	5 IU/mL		

REFERENCES:

1. Horellou M.H. Intérêt du dosage de la Protéine C dans les accidents thromboemboliques veineux. Feuil. Biol. 1985.
2. Stenflo J. Structure and Function of Protein C. Semin. Thromb. Haemostasis. 1984.
3. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
4. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

The following symbols may appear on the product labeling:

REF	Catalogue number	LOT	Batch code	RUO	Product for *in-vitro* research use, only
Rx	Numerical < x> identification of reagent		See instructions for use	WHO STD	WHO standard code
	Temperature limitation		Manufacturer		YYYY-MM-DD Use by
	Biological risks	→	Reconstitution volume	CONTENTS	Contents
Cx	Numerical < x> identification of control	i- MA	See instructions in Method Application guide	CONTAINS	Contains
EXP	Expiration date	Σ	Contains sufficient for <n> tests	UNIT	Measurement unit
TARGET VALUE	Target Value		Keep away from sunlight and heat	CALx	Numerical < x> identification of calibrator
ACCEPTANCE RANGE	Acceptance range				

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