LIAPHEN™ Free Protein S

REF 120004-RUO

R1 4 x 3 mL, R2 4 x 4.4 mL

Immuno-turbidimetric method for Free Protein S:Ag,
with ready to use liquid reagents.
FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

LIAPHEN™ Free Protein S kit is an immunoturbidimetric assay for in vitro quantitative determination of Free Protein S Antigen (Free PS:Ag) on human citrated plasma, using an automated method. Reagents are in the liquid presentation, ready to use.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:
Protein S (PS) is a vitamin K dependent glycoprotein, mainly synthesized in liver. Its concentration in normal human plasma is of about 25 µg/mL. About 40% (i.e. 10 µg/mL) is in the Free form and 60% (i.e. 15 µg/mL) circulates in blood as a non-covalent complex with C4b-BP. The balance between the free form and the C4b-BP bound form of protein S plays an important role, as only the Free Protein S is active through its anticoagulant activity as the cofactor of Activated Protein C.

PRINCIPLE:

LIAPHEN™ Free Protein S is an immunoturbidimetric method, based on antigen-antibody reaction: Free PS antigen of the sample reacts with Latex particles sensitized with two mouse monoclonal anti-Free PS antibodies, leading to latex particles agglutination. This agglutination can be directly detected by a change of absorbance. The absorbance change is directly proportional to the amount of Free PS:Ag in the sample.

REAGENTS:

R1 Reaction Buffer, liquid form.
4 vials of 3 mL.
R2 Latex, liquid form.
4 vials of 4.4 mL.

Reagents **R1** and **R2** contain BSA and small amounts of sodium azide (0.9 g/L).

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. 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.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

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:

- **4 months** at 2-8°C.
- **2 weeks** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole Buffer (AR021B-RUO/AR021K-RUO/AR021L-RUO/AR021M-RUO/AR021N-RUO), as diluent.
- Specific calibrator and controls with known Free PS:Ag titration, such as:

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

Also refer to the specific application guide of the analyzer used.

Materials:

- Spectrophotometer or automatic analyzer for immuno-turbidimetric assays.
- Calibrated pipettes; silicon glass or plastic test tubes.

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. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[1] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[1,2].

PROCEDURE:

The kit can be used for kinetics, automated methods. Perform the test at **37°C** and the turbidimetry is measured at **575nm** (other wavelengths can be used, preferentially between 540 and 800nm).

Assay method:

1. Reconstitute the reference preparation or plasma calibrator, and plasma controls as indicated in the specific instructions or according to internal practice.
Program the calibration concentrations from 0 to 150% Free PS:Ag (0-10-37.5-75-110-150% Free PS:Ag in Imidazole buffer), the **3:20** dilution corresponding to the indicated "C" concentration of Free PS:Ag for the commercial calibrator.

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

Specimens	Reference	Dilution
Controls	223201-RUO/ 223301-RUO	**3:20**
Specimen	n.a	**3:20**

Establish the calibration curve and test 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 schema 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 Imidazole buffer	10 µL
Imidazole buffer	10 µL
R1 Reaction Buffer	60 µL
Incubate **at 37°C** for **130 sec.**	
R2 Latex	100 µL
Mix and measure the optical density continuously (between **40 and 80 sec**) at **575nm,** at **37°C.**	

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

For high concentrations (between 150% and 300%), we recommend performing a pre-dilution in Imidazole buffer (the measured concentration should then be multiplied by the "pre-dilution" factor).

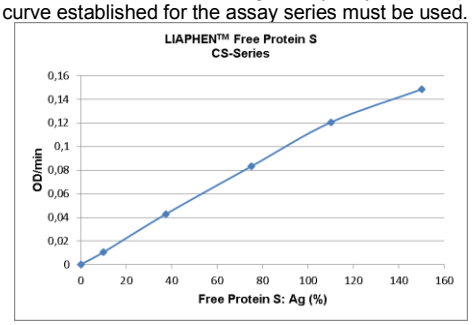
For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

CALIBRATION:

The LIAPHEN™ Free Protein S assay can be calibrated for the assay of Free PS:Ag in human plasma. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

- The calibration range is about 0 to 150%.

The calibration curve shown below is given by way of example only. The calibration curve established for the assay series must be used.



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:

- On Sysmex CS-series, the calibration curve (in point to point) is obtained with the ΔOD 575 nm along the Y-axis and the Free PS:Ag concentration, expressed as %, along the X-axis.
- The concentration of Free PS: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.

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 an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Heterophilic antibodies may interfere in the assay by giving abnormally high PS:Ag values.
- For the possible influence of Hook effect, refer to the specific application for the analyzer used (no significant effect is observed on Sysmex CS-series for Free PS:Ag concentrations up to 600%).

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<0.6% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 6 to 300% of Free PS: Ag on Sysmex CS-series, the test being linear up to 150% without redilution).
- Specificity: PS-deficient plasma was measured <6% on Sysmex CS-series. Addition of C4b-BP to plasma decreases the measured free PS:Ag concentration (increasing the amount of PS bound to C4b-BP).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	n	Mean	CV%	SD	n	Mean	CV%	SD
Normal	40	97.7	2.5	2.4	30	95.3	1.2	1.1
Pathological	40	29.0	1.8	0.5	30	29.9	1.9	0.6

- Correlation with reference method (INNOVANCE® Free PS Ag (Siemens) vs LIAPHEN™ Free Protein S on Sysmex CS-5100) :
  n = 131 y = 1.141x-7.982 r = 0.990
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	1000 mg/dL	**Heparin (UFH/LMWH)**	10/10 IU/mL
Bilirubin (Free)	60 mg/dL	**Rivaroxaban**	400 ng/mL
Bilirubin (Conjugated)	60 mg/dL	**Apixaban**	400 ng/mL
Intralipids	1000 mg/dL	**Dabigatran**	400 ng/mL
Fibrinogen	12 g/L	**Edoxaban**	400 ng/mL
Rheumatoid Factors	3000 IU/mL	**Platelets**	490.10^9/L

Also refer to the specific application guide of the analyzer used.

REFERENCES:

1. CLSI Document H21-A5: “Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline”. 2008.
2. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.