BIOPHEN™ α2-Antiplasmin (LRT)

CE

IVD

REF 220502

R1 R2 3 x 3 mL

Chromogenic method for the α2-Antiplasmin assay in plasma with ready to use liquid reagents.

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English, last revision: 01-2021

INTENDED USE:

BIOPHEN™ α2-Antiplasmin (LRT) kit is a chromogenic method for the *in vitro* quantitative determination of α2-Antiplasmin (α2-AP) activity, in human citrated plasma, using a manual or automated method. Reagents are in the liquid presentation, ready to use (LRT = Liquid reagent Technology).

SUMMARY AND EXPLANATION:

Technical:[1]

Alpha 2-Antiplasmin (or α2-Antiplasmin or plasmin inhibitor) is a serine protease inhibitor (serpin) responsible for inactivating plasmin, an important enzyme that participates in fibrinolysis and degradation of various proteins.

Clinical:[1-4]

Assaying α2-AP activity may be useful in case of α2-AP deficiency or during fibrinolytic therapy.

PRINCIPLE:

α2-AP present in the plasma sample inactivates plasmin. The residual plasmin cleaves the specific substrate SPm-41, releasing para-nitroaniline (pNA), which color is measured at 405nm. There is an inverse relationship between color development and α2-AP activity in the tested plasma.

α2-AP + Plasmin $_{excess}$ → [α2-AP-plasmin] + **residual plasmin**

Residual plasmin

SPm-41 ⟶ Peptide + pNA

REAGENTS:

R1 **Human Plasmin**, liquid form, ready to use. Contains small amounts of sodium azide (0.9 g/L).

R2 **Chromogenic substrate, specific for plasmin** (SPm-41), liquid form, ready to use. Contains Proclin.

R1 R2 **3 vials of 3 mL**

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the 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.
- 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* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize while avoiding formation of foam and load it directly on the analyzer following application guide instruction.

For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

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:

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

If the substrate becomes yellow, this indicate a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Diluent: Physiological Saline (0.9% NaCl) or Imidazole buffer (AR021B/AR021K/AR021L/AR021M/AR021N). Use the same buffer for all dilutions performed.
- Specific calibrator and controls titrated for α2-AP such as :

Product name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

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

Materials:

- Spectrophotometer or automatic analyzer for chromogenic assays.
- Stopwatch; Calibrated pipettes; 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[5] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references.[5]

PROCEDURE:

The kit can be used for kinetics, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity at **405nm**.

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

Assay method:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. Calibrators should be diluted in the diluent as described in the table below in order to prepare the calibration curve ("C" defines the concentration of α2-AP or by definition 100% for a normal plasma pool).

The calibration curve can be established using a commercial plasma calibrator with a known concentration of α2-AP (**C**) or using a pool of citrated normal plasmas (at least 30 normal individuals, men and women, aged between 18 and 55 years, with no known treatments or diseases), which, by definition, has a α2-AP titer of **100%**. The assay includes a **1:30** plasma dilution, which by definition, represents the **100%** α2-AP level or a concentration "**C**" for the commercial calibrator. In this case, the **150%** concentration (C1) is obtained by diluting this calibrator by the following dilution factor: **30 x (C): 150.**

Calibrator	C1	C2	C3	C4	C5	C6
α2-AP (%)	150	100	50	25	12.5	0
Volume of Calibrator	1500µL	660 µl of C1	500µL of C2	500µL of C3	500µL of C4	0
Volume of diluent	0 µL	330µL	500µL	500µL	500µL	500

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

Specimens	References	Dilution
Controls	223201 / 223301	1:30
Specimens	NA	1:30

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. In a plastic tube preincubated at **37°C**, introduce:

	Volume
Plasma to test, calibrator or controls **diluted**	200 µL
R1 Human plasmin preincubated at **37°C**	200 µL
Mix and incubate **at 37°C for 4 minutes exactly**, then introduce:	
R2 SPm-41 Substrate preincubated at **37°C**	200 µL
Mix and incubate at **37°C for 4 minutes exactly**	
Stop the reaction by introducing:	
Citric acid (2%)*	400 µL
Mix and measure the absorbance **at 405nm** against the corresponding blank.	

*Or acetic acid (20%). The yellow color is stable for 1 hour.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R2, R1, diluted plasma.

Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.
Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

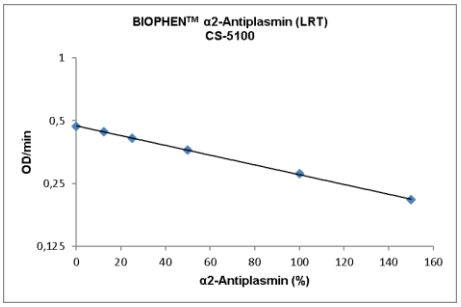
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ α2-Antiplasmin (LRT) assay can be calibrated for the assay of α2-AP activity. 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% (on Sysmex CS-series).

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:

- For the manual endpoint method, plot the calibration curve lin-log, with the OD 405 nm along the Y-axis and the α2-AP concentration, expressed as %, along the X-axis.
- When employing the kinetics method, use ΔOD 405 instead of OD 405.
- The concentration of α2-AP (%) 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 should be interpreted according to the patient's clinical and biological condition.

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.
- Specificity on samples with low concentration in α2-AP : α2-AP depleted plasma measured around 8-15%. A variant protocol using a shorter incubation time with plasmin (30 sec on Sysmex CS-series) promotes the activity of α2-AP and renders negligible the reactions of other inhibitors.

EXPECTED VALUES:

The α2-AP concentration in adults is usually expected between 75% and 135%[6,7]. However, each laboratory has to determine its own normal range.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<3.1% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 10 to 150% of α2-AP on Sysmex CS-series).
- 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 2 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	10	113.8	1.0	1.2	20	112.8	1.6	1.8
Abnormal	10	36.7	1.8	0.7	20	37.1	2.4	0.9

- Correlation with reference method (Berichrom A2antiplasmin vs BIOPHEN™ α2-Antiplasmin (LRT) on Sysmex CS-5100) :
  n = 60 y = 0.95x + 5.04 r = 0.995
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	Bilirubin (C/F)	Intralipids	Heparins (UFH/LMWH)
500 mg/dL	28 mg/dL	300 mg/dL	1 IU/mL

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

REFERENCES:

1. Carpenter S.L. and Mathew P. α2-Antiplasmin and its deficiency: Fibrinolysis out of balance. Haemophilia. 2008.
2. Kettle P. and Mayne E.E. A bleeding disorder due to deficiency of α2-Antiplasmin. J Clin Pathol. 1985.
3. Weitz J.I. *et al.* α2-Antiplasmin supplementation inhibits Tissue Plasminogen Activator-induced Fibrinogenolysis and bleeding with little effect on thrombosis. J clin Invest. 1993.
4. Aoki N. and Yamanaka T. The α2-Antiplasmin inhibitor levels in liver disease. Clin Chem Acta. 1978.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
6. Kratz A. *et al.* Laboratory Reference Values. The New England Journal of Medicine. 2004.
7. Andrew M. *et al.* Maturation of the hemostatic system during childhood. Blood. 1992.

SYMBOLS:

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

R2 H317 : May cause an allergic skin reaction.

Changes compared to the previous version.