BIOPHEN® α2-Antiplasmin (LRT)

Ref 220502

IVD

Chromogenic assay for measuring Alpha-2- Antiplasmin in plasma, designed with ready to use liquid reagents.

Not for Sale in the US

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INTENDED USE:

BIOPHEN® α2-Antiplasmin (LRT) kit is a chromogenic assay for the 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:

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

ASSAY PRINCIPLE:

α2-Antiplasmin present in the plasma sample inactivates plasmin (R1). The residual plasmin cleaves the specific substrate SPm-41 (R2), 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-Antiplasmin + Plasmin $_{excess}$ → [α2-Antiplasmin-plasmin] + **residual plasmin**

Residual plasmin

SPm-41 ⟶ Peptide + pNA

REAGENTS:

R1: Reagent 1: Human Plasmin (liquid form, ready to use). 3 vials of 3 mL each.

R2: Reagent 2: Chromogenic substrate, specific for plasmin (SPm-41) (liquid form, ready to use). 3 vials of 3 mL each.

CAUTIONS AND WARNING:

- Any product of biological origin must be handled with all the required cautions, as being potentially infectious.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- Use only reagents from kits with the same lot number.
- The plasmin concentration may present variations from lot to lot, but it is exactly adjusted for each new lot of reagent.
- Reagent contains low concentration of Sodium azide (0.9 g/L) which may react with lead and copper plumbing to form highly explosive metal azides. Dispose of properly in accordance with local regulation.
- The disposal of waste materials must be carried out according to current local regulations.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- For in vitro diagnostic use.

PREPARATION AND STABILITY OF REAGENTS:

R1: Reagent 1: **Human plasmin**

(Clear vial) Ready to use. Let homogenize for 30 minutes at room temperature (18-25°C) before use. Homogenize well before each use, taking care of product viscosity, as well as when pipetting for manual method.

Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

- **5 weeks** at 2-8°C.
- **7 days** at room temperature (18-25 °C).
- **Do not freeze.**

R2: Reagent 2: **Chromogenic substrate (SPm-41)**

(Brown vial). Ready to use. Let homogenize for 30 minutes at room temperature (18-25°C) before use. Homogenize well before each use.

Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

- **5 weeks** at 2-8°C.
- **7 days** at room temperature (18-25 °C).
- **Do not freeze.**

Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.

STORAGE CONDITIONS:

Reagents must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the box.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method)
- Imidazole Buffer (ref AR021) or appropriate dilution buffer. Alternatively physiological saline (0.9% NaCl) could be used.
- Plasma Calibrator with a known α2-AP activity
  Or Reference material for α2-AP
  Or Normal citrated reference human plasma pool.
- Normal and Abnormal Control Plasmas with a known α2-AP activity.

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays, with a wave-length set up at 405 nm.
- Stop watch.
- Calibrated pipettes.

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations (In the USA, refer to NCCLS/CLSI document for further instructions on specimen collection, handling and storage).

- Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood.

- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M) in order to avoid any activation, through a net venipuncture. The first tube must be discarded.

- Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

- Storage of plasma:
  - 4 hours at room temperature (18-25°C)
  - 1 month at -20°C.
  - 18 months at -70°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

The BIOPHEN® α2 Antiplasmin (LRT) kit can be used for kinetics methods, automated on instruments, and can also be used for end point methods. The assay is performed **at 37°C** and the color developed is measured **at 405 nm**.

Automated methods:

Applications to the various analyzers are available upon request. **Refer to each specific application and specific cautions for each instrument.**

Assay (manual method):

Dilute the tested samples, calibrators and the controls solutions **1:30 in imidazole buffer**.

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

	Test tube
Plasma to test, calibrator or control (**diluted 1:30**)	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:	
Acetic acid (20%)	400 µL
Mix and measure the absorbance **at 405nm** against the corresponding blank	

The yellow color is stable for at least 1 hour.

*The sample blank is obtained by mixing the reagents in the reverse order from that of the test i.e.: Acetic acid (20%), SPm41 substrate (R2), Plasmin (R1), diluted plasma. Measure the absorbance **at 405 nm**. The sample blank value must be deduced from the absorbance measured for the corresponding assay.*

If higher or lower reactive volumes than those indicated here above are required for the method used, the same respective proportions between reagent concentrations and volumes used, must be adhered to, in order to maintain the assay performances.

CALIBRATION:

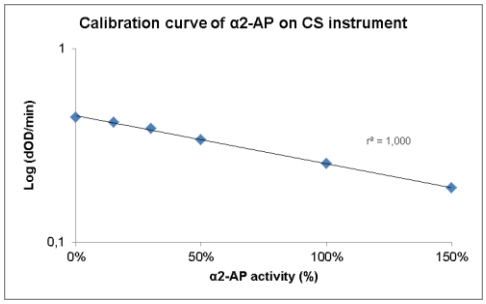
Calibration can be performed with a commercially available plasma calibrator with a known α2-Antiplasmin concentration **(C),** or a normal pooled human citrated plasma (made with plasmas from at least 30 normal individuals, males or females, aged between 18 and 55 years, and out of any medication or disease), with the assigned value of **100%** α2 Antiplasmin.
The assay includes a standard plasma dilution of **1:30**. By definition, this latter dilution represents the **100 %** α2-AP activity for the pool or the indicated **"C"** concentration for the commercial calibrator.
In this case, the **150%** concentration (C1) is obtained (in the assay conditions) by using the following dilution factor: **30 x C: 150.**

The following calibration range must be prepared as follows **from prediluted calibrator at C1 concentration:**

Cal	C1	C2	C3	C4	C5	C6
% α2 Antiplasmin	150	100	50	25	12.5	0
Vol of Plasma calibrator (µl)	1500µL	660 µl of C1	500µL of C2	500µL of C3	500µL of C4	0
Vol of Imidazole Buffer µl	0 µL	330µL	500µL	500µL	500µL	500

In order to get the full assay performances, the calibration curve must be prepared just before running the assay.

The calibration curve below is given for example only, using the CS instrument. Only the calibration curve generated for the series of assays performed should be used for calculating the concentrations in the assayed samples.



QUALITY CONTROL:

Using suitable commercially available quality control plasmas, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents.
At least one quality control at each level in each series, as per good laboratory practice, should be included to valid it. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents, or after each important analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method.
Each laboratory should establish and verify its own target values, acceptance ranges and expected performances, according to the instruments and protocols used.

RESULTS:

- For the end-point method, use a **lin-log** graph paper and plot on abscissae the α2-Antiplasmin concentrations **(%)** and on ordinates the corresponding absorbances **A405(Log**).
- Alternatively, statistics software can be used for establishing the dose response calibration curve. A semi-log inverse linear relationship is obtained between α2-AP concentration and absorbance (A405).
- Draw the calibration curve obtained.
- Calculate the "r²" value. Calibration is acceptable if **r² ≥ 0.98**, and if measured values for controls are in compliance.
  Using the manual method (test tube), A405 value is usually of about 2.0±0.3 for 0% α2-AP concentration. Indicatively, for the microplate method, A405 is expected lower than using the test tube method. A405 values can differ according to the instrument application used.
- The α2-AP concentration in the tested sample is directly obtained on the calibration curve (concentration corresponding to the measured A405). Results are expressed as % α2-AP.
- Using automated methods, the α2-AP concentrations are directly calculated by the analyser, respectively to the calibration curve, and the sample dilution used.
- The dynamic range is from **10 to 150% α2-AP.**

When the assay dilution is **1:30**, the α2-AP concentration is directly read on the calibration curve. When predilutions are used, multiply the measured α2AP concentration by the predilution factor in order to get the concentration in the tested specimen.

LIMITATION:

- No significant interference is observed for bilirubin concentrations < 28 mg/dL, haemoglobin concentrations < 500 mg/dL and triglycerides concentrations <300 mg/dL on CS5100 instrument, and heparin concentrations < 2 IU/mL (by spiking experiment in plasma). Some analytes can interfere in absorbance readings: in these cases, individual plasmas blanks are necessary when end-point manual methods are used.
- In order to get the optimal assay performances, the working instructions must be carefully observed. Each laboratory should verify performances in its exact working conditions.
- A lack of specificity can be observed in samples with low concentration in α2-AP (an α2-AP depleted plasma gives around 8-15% of α2-AP activity). A variant protocol using a shorter incubation time with plasmin (eg 30 sec on CS) can promote the activity of α2-AP and render negligible the reactions of other inhibitors.

EXPECTED VALUE:

By definition, the 100 % α2-AP concentration corresponds to the concentration in a normal human citrated plasma pool, obtained by pooling plasmas from healthy males or females aged from 18 to 55 years, and out of any medication or disease. The α2-Antiplasmin concentration in adults is usually expected from about 75% up to about 135%; each laboratory should establish its own reference interval in its exact working conditions.
The results are to be interpreted according to the patient's clinical and biological states.

PERFORMANCE:

- **Dynamic range**: **10 to 150 % α2-AP.**
- The detection threshold is calculated by measuring the "apparent" A405 obtained for a α2-Antiplasmin deficient sample less 2 standard deviations (SD). This **detection threshold is ≤10% on CS instrument** (as an example about 2.8% A2AP was obtained for one lot on CS5100).
- **Specificity**: An α2-Antiplasmin poor plasma was measured **< 25%** α2-AP.
- Example of reproducibility data obtained with normal control and abnormal control, using CS5100 instrument:

Sample	Intra Assay CV%				Inter Assay CV%			
	Normal control		Abnormal control		Normal control		Abnormal control	
A2-AP	N	10	N	10	N	10	N	10
	CV%	0.8	CV%	1.2	CV%	1.3	CV%	2.4

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5. Woodhams B, Girardot O, Blanco M-J, Colesse G, Gourmelin Y.Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001. Vol 12, No 4. 229-236.
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SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1