BIOPHEN™ AT anti-(h)-Xa LRT

REF 221123-RUO R1 R2 3 x 3 mL

REF 221127-RUO R1 R2 4 x 7.5 mL

Chromogenic method for the assay of Antithrombin in plasma, with ready to use liquid reagents.

FOR RESEARCH USE ONLY.

DO NOT USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

The BIOPHEN™ AT anti-(h)-Xa LRT kit is a chromogenic method for the *in vitro* quantitative determination of Antithrombin (AT) activity on human citrated plasma using an anti-Xa method, manual or automated.
Reagents are in the liquid presentation, ready to use (LRT, Liquid reagent Technology).
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:
AT is the major physiological coagulation inhibitor. It inhibits coagulation serine esterases, especially Thrombin, Factor Xa (FXa) and Factor IXa, regulates coagulation pathway and prevents from thrombosis. When complexed to heparin, AT becomes a potent and fast acting inhibitor of coagulation serine esterases[1].

PRINCIPLE:

BIOPHEN™ AT anti-(h)-Xa LRT assay is a kinetics method based on the inhibition of FXa, which is at a constant concentration and in excess, by AT, in presence of heparin. The remaining FXa is then measured by its amydolitic activity on a FXa specific chromogenic substrate (SXa-11-65), which releases pNA. The amount of pNA generated is inversely proportional to the AT concentration present in the tested plasma.

Heparin + AT ➔[AT Hep.]
[AT Hep.] + [Excess FXa] ➔ [FXa-AT-Hep.] + [Remaining FXa]
[Remaining FXa] + SXa-11-65 ➔ Peptide + pNA

REAGENTS:

R1 **Human Factor Xa,** liquid form, at pH about 7.85. Contains heparin, BSA and small amounts of sodium azide (0.9 g/L).
R2 **Factor Xa substrate**, Chromogenic substrate, specific for Factor Xa (11-65), liquid form with stabilizers. Contains Proclin.

REF 221123-RUO ➔ R1 R2 **3 vials of 3 mL.**
REF 221127-RUO ➔ R1 R2 **4 vials of 7.5 mL.**

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 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* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize, 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 (taking care of product viscosity).

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.**

A yellow color indicates a contaminated substrate. 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-RUO/AR021K-RUO/AR021L-RUO/AR021M-RUO/AR021N-RUO). Use the same buffer for all dilutions performed.
- Specific calibrators and controls with known 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 instrument for chromogenic assays.
- Stopwatch; 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[2] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[2,3].

PROCEDURE:

The kit can be used for kinetic, 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 below in order to prepare the calibration curve ("C" defines the concentration of AT):

When the calibration curve is established using a commercial calibrator plasma (e.g.: BIOPHEN™ Plasma Calibrator), the **1/50** dilution corresponds to the indicated concentration (**C**) of AT and the **1/33.3** dilution to 1.5 fold this concentration. For a calibrator with a titer of C, the **150%** level (under assay conditions) is obtained by diluting this calibrator by the following factor: **33.3x(C)/100**.
The calibration curve can also be established 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 AT titer of **100%.** The assay includes a **1/50** plasma dilution, which by definition, represents the **100%** of activity. The dynamic calibration curve ranges from about 10 to 150% AT. The **1/33.3** dilution of the pool in the diluent represents **150%** of AT activity.

The following calibration range can then be prepared as follows (from prediluted calibrator or pool):

AT (%)	0	C:8	C:4	C:2	C	3C:2
AT (%)	0	12.5	25	50	100	150
Volume Calibrator	0µL	60µL	125µL	250µL	500µL	Obtained by dilution factor : **33.3 x C:100 in diluent**
Volume Diluent	500µL	420µL	375µL	250µL	0µL	

Prepare the calibration curve just before running the assay for optimal performances.

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

Specimens	Reference	Dilution
Controls	223201-RUO / 223301-RUO	**1 :50**
Specimens	NA	**1 :50**

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. Dispense the following to a plastic tube incubated at **37°C**:

	Volume
Specimen, control or calibrator diluted	200 µL
R1 **Human Factor Xa** Pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for 1 minute**, then add the following:	
R2 **Factor Xa substrate** Pre-incubated at **37°C**	200 µL
Mix and incubate at **37°C** for **1 minute exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density 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, dilute specimen.
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 this latter is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

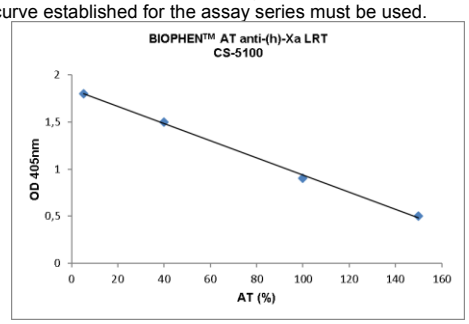
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ AT anti-(h)-Xa LRT assay can be calibrated for the assay of AT 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.
Using a linear scale:

- The calibration range is about 5 to 150% (on CS-5100).

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 defined, 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 acceptable range for the method.
Each laboratory must define its acceptable 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 405 nm along the Y-axis and the AT concentration, expressed as %, along the X-axis.
- The concentration of AT (%) 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.
- As the assay is an Anti-Xa method, there is no expected interference of Heparin Cofactor II, ∝2-macroglobulin or ∝1-Antitrypsin[4].
- Direct Factor Xa Inhibitors (DOACs) may induce an overestimation of measured AT activity.

PERFORMANCE:

- The lower analyzer detection limit depends on the analytical system used (<5% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 5 to 200% of AT on Sysmex CS-5100).
- The BIOPHEN™ AT anti-(h)-Xa LRT assay is insensitive to heparin at usual concentrations.
- Specificity: AT poor plasma was measured ≤15%.
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 20-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
Control 1	40	34,7	1,8	0,6	120	34,6	3,1	1,1
Control 2	39	89,7	1,2	1,1	120	87,7	2,3	2,0

- By the assay principle, no interference to anti-IIa anticoagulants, such as Dabigatran or Bivalirudin, is expected.
- Correlation with reference method (INNOVANCE Antithrombin (Siemens) on CS-5100) :
  n = 120 y = 1,04x – 0,49 r = 0,945
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations :

Intralipids (mg/dL)	Hemoglobin (mg/dL)	Bilirubin (F/C) (mg/dL)	Heparin (UFH/LMWH) (IU/mL)
1000	1000	60	2

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

REFERENCES:

1. Mann K.G. Biochemistry and Physiology of blood coagulation. Thrombosis and Haemostasis. 1999.
2. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
3. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.
4. Odegard O R *et al.* Heparin cofactor activity measured with an amidolytic method. Thromb res 6. 1975.

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.