



Manufactured By: HYPHEN BioMed

BIOPHEN H-CoII Technical File

(#A221902)

Chromogenic assay for the quantitative measurement of Heparin Cofactor II in human citrated plasma or in purified systems.

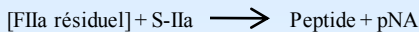
Assay range: 0 - 100% Heparin Co. II

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Assay principle

- BIOPHEN H-CoII kit is a chromogenic assay for measuring the Heparin Cofactor II activity in human plasma or in purified systems, using a chromogenic method, manual or automated.
- Heparin cofactor II is an anticoagulant protein which inhibits specifically thrombin. This inhibition is strongly enhanced and speeded up by binding to glycoaminoglycans such as dermatan sulfate (1).
- The BIOPHEN H-CoII assay is a chromogenic method based on the inhibition of a constant and in excess amount of thrombin, by the tested heparin cofactor II in presence of exogenous dermatan sulfate, and hydrolysis of a Thrombin specific chromogenic substrate (SIIa-01), by thrombin in excess. pNA is then released from the substrate. The amount of pNA released is then a relation of the residual thrombin activity. There is an inverse relationship between the concentration of heparin cofactor II and color development, measured at 405 nm.



Intended use: For in vitro research use only RUO

- Measurement of Heparin Cofactor II concentrations in plasma or in purified fractions.

Kit presentation: 2 x 50 tests (microplate)

R1: Reagent 1: Reaction Buffer: Assay reaction buffer (Tris-NaCl-BSA at pH 8.0), ready to use (2 vials).

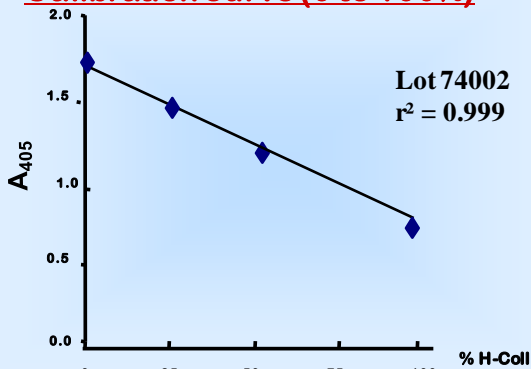
R2: Reagent 2: Highly purified Human Thrombin, containing dermatan sulfate, lyophilised (2vials).

R3: Reagent 3: SIIa-01 chromogenic substrate, lyophilised (2 vials).

Procedure

- Specimen: citrated human plasma or purified Heparin Cofactor II.
- Plasma Dilution: **1:50**.
- Calibration: Plasma calibrator, plasma pool or HCoII standard.
- End-point method or kinetics protocols.

Calibration curve (0 to 100%)

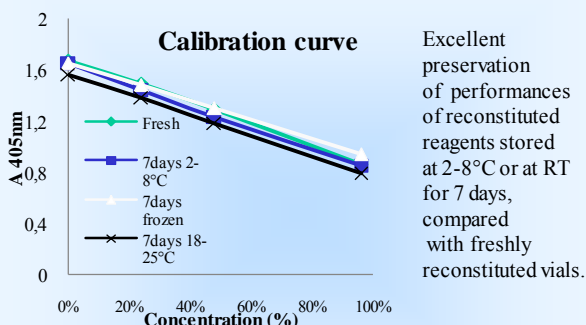


The assay has a dynamic range from 0 to 100% of H-CoII.

Assay Characteristics

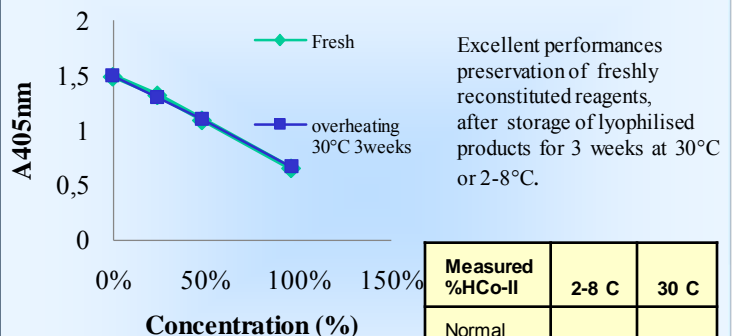
- Total assay time : about **5 minutes**
- Assay range : **0 to 100 %** of Heparin Cofactor II in plasma (or **0 to 200%** with plasma dilution **1:100**)
- Reproducibility: $\leq 3 \%$ ($N \geq 5$ R1 or R2, tested by manual method on the point 100% H-CoII)
- Detection limit (blank-3SD, $N \geq 10$): **< 4 %** (specification $\leq 5\%$)
- Can be used with: manual, automated, and microplate methods.
- Inter-assay performances (CV, $N \geq 10$) : **< 7 %**
- Intra-assay performances (CV, $N \geq 10$) : **< 5 %**

Stability of reconstituted reagents



Measured % H-CoII	Fresh	7d/2-8 C	7d/RT
Normal control	81%	83%	82%
Abnormal control	53%	54%	54%

Overheating study



Measured %HCo-II	2-8 C	30 C
Normal control	76%	79%
Abnormal control	47%	52%

Kits can be shipped at RT for a short period without damage.

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ATIII and Heparin interference

Goal: To check that there is no significant interference of heparin concentrations in plasma, up to 2IU/ml, or ATIII concentrations up to 150µg/ml, on Heparin Cofactor II measurement using the BIOPHEN H-CoII assay.

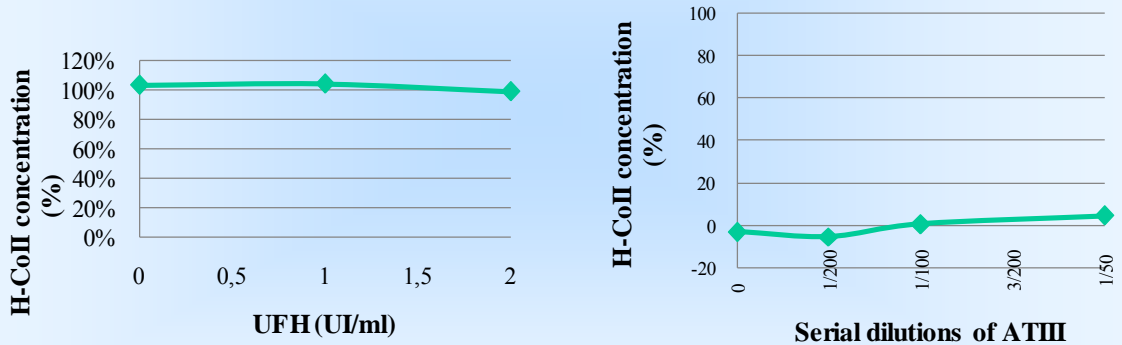
Material and tested samples:

-Biophen H-CoII: lot 74002

-Normal Plasma pool supplemented with Heparin Choay (UFH), from 0 to 2 IU/ml.

-Human ATIII highly purified, lyophilised, reconstituted and diluted to 150µg/ml in TBSA buffer, then diluted at 1/50, 1/100, 1/200, 0 in R1 buffer.

Results:

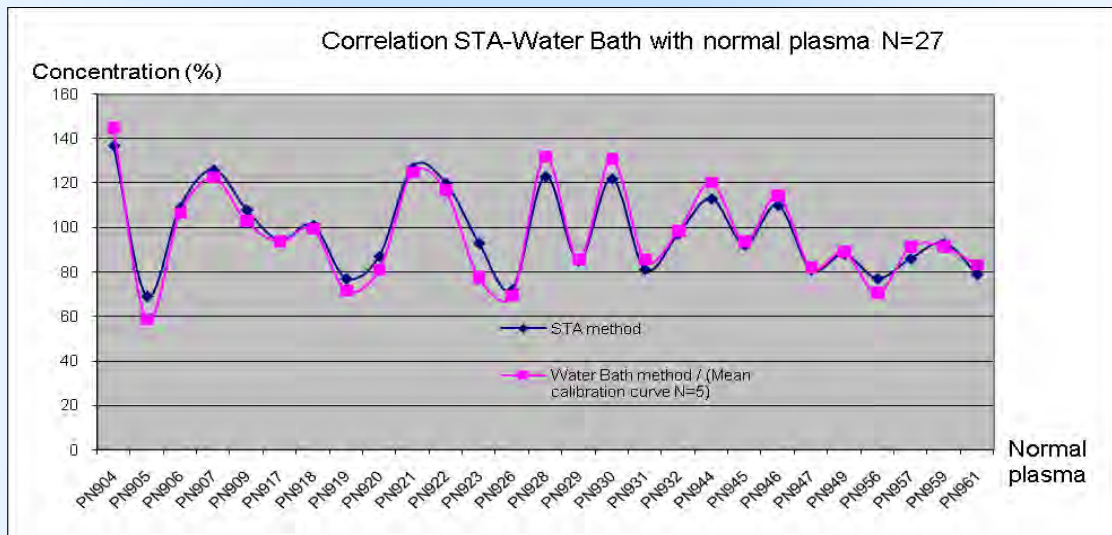


Conclusions: No significant interference of heparin up to 2 IU/ml in plasma; no significant interference of ATIII.

STA-R method vs Water-Bath method

Goal: Comparison of Normal plasma (N=27) assayed using the Water-Bath or STA-R method.

Mean conc. (%)	98	98
Maximum	137	145
Minimum	69	59
SD	19	21
Mean-2SD	61	55
Mean+2SD	135	141
Normal range	[61-135]	[55-141]
Median	93	94



Conclusions: Good consistency of the results obtained using the Water Bath or STAR method. The normal range using Biophen HCoII assay is of from about 60 to 140%, in compliance with literature data.

Microplate method

Goal: Validation of using the BIOPHEN H-CoII Kit with a microplate method.

Material and tested samples:

-Biophen H-CoII: lot 74002

-Calibrator : lot 70601, Normal control : lot 63004, Abnormal control : lot 71601.

Results :

H-CoII Concentration (%)	OD 405nm microplate	OD 405nm water-bath method
96	0,40	0,83
48	0,56	1,23
24	0,68	1,47
0	0,74	1,65
R²	0,993	0,999
Normal Control lot 63004 (target value =81%±10%)	83,80%	81,00%
Abnormal Control lot 71601 (target value=51%±10%)	56%	52,00%

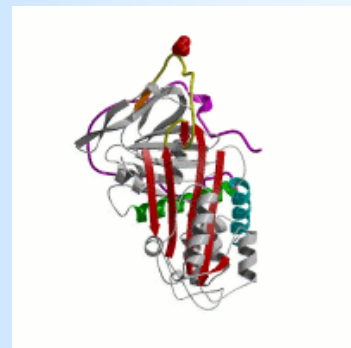
Conclusion : Good linearity (>0.99) and coherent control values in two independent series validates the Microplate method .

Biochemistry

- Heparin Cofactor II (heparin cofactor A, antithrombin BM, dermatan sulfate cofactor, human leuserpin-2) is a single chain glycoprotein (Mr=65.600, 10% carbohydrate, pI=5.05) member of the serine protease inhibitor (serpin) family.

- In contrast to antithrombin III, the only coagulation protease inhibited by HCII is thrombin (1).

-Dermatan sulfate increases the thrombin inhibition rate by HCII about 1300-fold, from $5.0 \times 10^5 \text{ M}^{-1} \text{ min}^{-1}$ in the absence of glycosaminoglycan to $6.4 \times 10^8 \text{ M}^{-1} \text{ min}^{-1}$ at optimal concentrations of dermatan sulfate. In contrast, the inhibition rate of thrombin by ATIII remains low ($< 1 \times 10^7 \text{ M}^{-1} \text{ min}^{-1}$) even in the presence of this glycoaminoglycan, Furthermore thrombin becomes complexed exclusively with HCII in the presence of $\geq 100 \mu\text{g/ml}$ dermatan sulfate (7).



Heparin Cofactor II

Clinical applications

- Heparin Cofactor II activity in plasma may be a predictive factor of atherosclerotic changes, especially in elderly individuals (3).

- Heparin Cofactor II might inhibit thrombosis after endothelial injury (1).

- High plasma Heparin Cofactor II activity protects from restenosis after femoropopliteal stenting (8).

References

1. Li He et al. Heparin Cofactor II inhibits arterial thrombosis after endothelial injury. J. Clin. Invest. 109:213-219 (2002)
2. Tollefsen DM et al. Heparin Cofactor II activity in patients with disseminated intravascular coagulation and hepatic failure. Blood 66:769-774 (1985)
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5. Tollefsen DM et al. Heparin cofactor II deficiency. Arch. of Path. And Lab. Med. 126:1394-1400 (2002)
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7. Tollefsen DM et al. Activation of Heparin Cofactor II by dermatan sulfate. J. Biol. Chem. 258:6713-6716 (1983)
8. Schillinger et al. High plasma heparin cofactor II activity protects from restenosis after femoropopliteal stenting Thromb Haemost 92 :1108-1113 (2004)