



HYPHEN BioMed
155 rue d'Eragny
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France



ANALYSIS CERTIFICATE

BIOPHEN™ Anti-IIa (2 stages Heparin assay) - #220005

Lot: F2001200

QC Release: 2020-10-26

Expiration date: 2023-02-28

Components	Qty	Exp. (months)	Lot #	Exp. date
R1 : ATIII(h)	2 vials	30	F201501200	2023-03-17
R2 : Thrombin	2 vials	30	F201501200	2023-03-20
R3 : Substrate	2 vials	30	F201501200	2023-02-28

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Analytical data	Specifications
<p>1. ATIII (h)</p> <p>a. Lot homogeneity (A405 for 0 IU/ml heparin) N= 25 Mean (A405): 2.795 CV: 0.8 %</p> <p>b. AT content per vial (anti-Xa activity chromogenic assay on raw material) 1.72 IU</p> <p>c. Indicative AT content per vial (A280nm/Lowry on raw material) 190 µg</p> <p>d. SDS PAGE (on raw material) 1 major band of about 58,000 Da</p> <p>e. Absence of heparin Absence</p>	<p>≤ 5 %</p> <p>≥ 1.2 IU</p> <p>1 major band of about 58,000 Da</p> <p>Absence</p>
<p>2. Thrombin (h)</p> <p>a. Lot homogeneity (A405 for 0 IU/ml heparin) N=25 Mean (A405): 2.807 CV: 1.2 %</p> <p>b. SDS PAGE (5% acrylamide) (on raw material) 1 major band of about 35,000 Da</p> <p>c. Indicative IIa content per vial (Lowry on raw material) -36 µg</p> <p>d. Indicative clotting activity per vial (clotting assay on raw material) (respectively to the WHO/NIBSC standard for h(IIa)) 110 NIH (or IU)</p> <p>e. Indicative specific chromogenic activity (on raw material) (chromogenic assay using CS-01(38)) 5.98 nkats/µg</p>	<p>≤ 5 %</p> <p>1 major band of about 35,000 Da</p>
<p>3. Thrombin substrate</p> <p>a. Blank value N= 5 Mean (A405): 0.108</p> <p>b. Lot homogeneity (A405 for 0 IU/ml heparin) N= 25 Mean (A405): 2.786 CV: 1.2 %</p> <p>c. Indicative content per vial (raw material) 6.25 mg (about 11.3 µmol)</p> <p>d. HPLC analysis purity grade (raw material) 98.7 %</p> <p>e. Experimental molecular weight (raw material) 552 Da</p>	<p>N≥5 A405 ≤ 0.30</p> <p>≤ 5 %</p> <p>≥ 95%</p> <p>553 ± 5 Da</p>

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5. <u>Assay reactivity (in the tested dilution, CS-series)</u>								$R^2 \geq 0.98$ $\Delta A405 (0-0.04) \geq 0.65$
IU/mL	0.0	0.005	0.01	0.02	0.03	0.04	R ²	
A405 UFH purified	1.563	1.254	1.038	0.694	0.462	0.319	1.000	
A405LMWH purified	1.531	1.145	0.835	0.413	0.217	0.131	0.998	

6. <u>UFH Detection threshold (concentration in the tested dilution, CS-series)</u>		≤ 0.005 IU/mL
In Purified solution	< 0.005 IU/mL	

7. <u>Stability of reconstituted reagents (purified milieu, CS-series)</u>									$R^2 \geq 0.98$ $\Delta A405 (0-0.04) \geq 0.65$ $\Delta A405 (0 IU/mL) \leq 10\%$ between fresh and stored $A405 (Free pNA) \leq 0.30$
Reagents tested after 15 days at 2-8°C, or 4 days at RT (18-25°C) or Frozen ≤ -20°C									
A405 values for UFH									
UFH IU/mL	0.0	0.005	0.01	0.02	0.03	0.04	R ²	A405 (Free pNA)	
Freshly restored	1.530	1.225	1.006	0.669	0.451	0.319	0.998	0.115	
15 days at 2-8°C	IN PROGRESS								
4 days at RT	1.511	1.248	1.051	0.735	0.524	0.385	0.998	0.113	
Frozen ≤ -20°C	IN PROGRESS								

Comments :	<input checked="" type="checkbox"/> PASSED IN COMPLIANCE
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Date: 2020-10-26

QC Manager: S. LECOURT

