



HYPHEN BioMed
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France



ANALYSIS CERTIFICATE

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

Lot: F2000195

QC Release: 2020-03-19

Expiration date: 2022-08-01

Components	Qty	Exp. (months)	Lot #	Exp. date
R1 : ATIII(h)	2 vials	30	F201400195	2022-08-01
R2 : Thrombin	2 vials	30	F201400195	2022-08-07
R3 : Substrate	2 vials	30	F201400195	2022-08-01

Beo

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Analytical data	Specifications
<p>1. <u>ATIII (h)</u></p> <p>a. Lot homogeneity (A405 for 0 IU/ml heparin)</p> <p style="margin-left: 40px;">N= 25 Mean (A405) : 2.928 CV : 1.4 %</p> <p>b. AT content per vial (anti-Xa activity chromogenic assay on raw material)</p> <p style="margin-left: 40px;">1.47 IU</p> <p>c. Indicative AT content per vial (A280nm/Lowry on raw material)</p> <p style="margin-left: 40px;">190 µg</p> <p>d. SDS PAGE (on raw material)</p> <p style="margin-left: 40px;">1 major band of about 58 000 Da</p> <p>e. Absence of heparin</p> <p style="text-align: center;">Absence</p>	<p style="text-align: center;">≤ 5 %</p> <p style="text-align: center;">≥ 1.2 IU</p> <p style="text-align: center;">1 major band of about 58,000 Da</p> <p style="text-align: center;">Absence</p>
<p>2. <u>Thrombin (h)</u></p> <p>a. Lot homogeneity (A405 for 0 IU/ml heparin)</p> <p style="margin-left: 40px;">N= 20 Mean (A405) : 2.924 CV : 2.0 %</p> <p>b. SDS PAGE (5% acrylamide) (on raw material)</p> <p style="margin-left: 40px;">1 major band of about 35 000 Da</p> <p>c. Indicative IIa content per vial (Lowry on raw material)</p> <p style="margin-left: 40px;">~27 µg</p> <p>d. indicative clotting activity per vial (clotting assay on raw material) (respectively to the WHO/NIBSC standard for h(IIa))</p> <p style="margin-left: 40px;">120 NIH (or IU)</p> <p>e. indicative specific chromogenic activity (on raw material) (chromogenic assay using CS-01(38))</p> <p style="margin-left: 40px;">4.94 nkats/µg</p>	<p style="text-align: center;">≤ 5 %</p> <p style="text-align: center;">1 major band of about 35,000 Da</p>
<p>3. <u>Thrombin substrate</u></p> <p>a. Blank value</p> <p style="margin-left: 40px;">N= 5 Mean (A405) : 0.113</p> <p>b. Lot homogeneity (A405 for 0 IU/ml heparin)</p> <p style="margin-left: 40px;">N= 20 Mean (A405) : 2.802 CV : 2.1 %</p> <p>c. Indicative content per vial (raw material)</p> <p style="margin-left: 40px;">6.25 mg (about 11.3 µmol)</p> <p>d. HPLC analysis purity grade (raw material)</p> <p style="margin-left: 40px;">98.7 %</p> <p>e. Experimental molecular weight (raw material)</p> <p style="margin-left: 40px;">553 Da</p>	<p style="text-align: center;">N≥5 A405 ≤ 0.30</p> <p style="text-align: center;">≤ 5 %</p> <p style="text-align: center;">≥ 95%</p> <p style="text-align: center;">553 ± 5 Da</p>

Geo

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Analytical data								Specifications
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	1.625	1.371	1.154	0.797	0.534	0.371	1.00	
A405LMWH purified	1.589	1.246	0.927	0.445	0.231	0.153	0.99	

6. <u>UFH Detection threshold (concentration in the tested dilution, CS-series)</u>		≤ 0.005 IU/mL
In Purified solution	< 0.001 IU/mL	

7. <u>Stability of reconstituted reagents (purified milieu, CS-series)</u>									$R^2 \geq 0.98$ $\Delta A405 (0-0.04) \geq 0.65$ $\Delta A405 (0 \text{ IU/mL}) \leq 10\%$ between fresh and stored $A405 (\text{Free pNA}) \leq 0.30$
Reagents tested after 15 days at 2-8°C, or 4 days at RT (18-25°C) or Frozen $\leq -20^\circ\text{C}$									
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	1.557	1.146	0.897	0.506	0.302	0.196	0.998	0.106	
15 days at 2-8°C	1.535	1.175	0.948	0.582	0.371	0.255	0.998	0.105	
4 days at RT	1.575	1.209	0.973	0.609	0.395	0.268	0.998	0.104	
Frozen $\leq -20^\circ\text{C}$	1.519	1.112	0.864	0.486	0.288	0.184	0.998	0.106	

Comments :	<input checked="" type="checkbox"/>	PASSED IN COMPLIANCE

Date: 2020-03-19

QC Manager: S. LECOURT

P.O.
