

Application Sheet for Antithrombin with HEMOSTAT Antithrombin^{liquid}

HumaClot Junior (model HC1) **REF 18680**
HumaClot Duo Plus (model HC2) **REF 15650**
HumaClot Quattro **REF 15660**

The parameters defined in this application sheet have been developed to provide optimal product performance with the assay and instrument combination. Any modification to these parameters may affect the performance of this and other assays in use on your system and the resulting assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. The application sheet lists all combinations of controls and calibrators for use with the reagent and instrument system; other combinations are not validated or supported.

For additional information, please refer to the respective User Manual of the instrument and check current instructions for use (IFU) for reagents, controls, calibrators and tables of assigned/analytical values.

Typical performance data can be found in the Verification Report of the respective instrument, accessible via

www.human.de/data/gb/vr/18680.pdf
www.human.de/data/gb/vr/15650.pdf
www.human.de/data/gb/vr/15660.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Material Required

Material	REF	Size	On-Board Position
HEMOSTAT Antithrombin ^{liquid}	36102		
RGT Antithrombin Reagent		4 x 3 ml	Heated reagent position (on HumaClot Duo Plus and HumaClot Quattro)
SUB Substrate		2 x 3 ml	Heated reagent position
CAL HEMOSTAT Calibrator	35500	4 x 1 ml	-
CPN HEMOSTAT Control Plasma Normal	35001	6 x 1 ml	-
CPA HEMOSTAT Control Plasma Abnormal	35002	6 x 1 ml	-
NaCl 0.9% Sodium chloride	-	-	for dilution of calibrator, controls and samples
Cuvettes with pre-filled mixers	15660/10	5 x 100 pcs	Pre-heated cuvette positions
Cuvette bag with separate mixer	15660/11	500 pcs	
Cuvette bag with separate mixer	15660/12	5 x 500 pcs	
Reducer Ring	15660/52	2 pcs	(Standard accessory HumaClot Duo Plus / Quattro)

Pipetting Scheme

Sample Pre-Dilution (1:11)	
Sample	10 µl
0.9 % NaCl (0.154 mol/l)	100 µl
Pipetting	
<i>HumaClot Duo Plus and HumaClot Quattro: pre-warm [RGT], [SUB] and cuvettes at 37 °C. HumaClot Junior: pre-warm [SUB] and cuvettes at 37 °C.</i>	
1 pre-diluted sample	33 µl
[RGT] Antithrombin reagent	105 µl
<i>Transfer cuvette with sample and [RGT] into the measuring channel</i>	
Incubation time	180 s
Start Reagent [SUB] Substrate	30 µl
Auto start	yes

Standard Curve Calibration

A new standard curve must be established when

- changing to a new HEMOSTAT of Antithrombin^{liquid} LOT
- after major maintenance or service
- if indicated by quality control results
- when required by laboratory control procedures and/or governmental regulations.

The following steps have to be followed:

Reconstitution of the HEMOSTAT Calibrator ([REF] 35500) with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).

Find the LOT-specific AT %-value in the analytical value sheet of the HEMOSTAT Calibrator [CAL].

- Calculate the respective AT %-value for Cal 2, Cal 3 and Cal 4 based on a serial dilution (1:2, 1:4, 1:8) of Cal 1 (analytical value of HEMOSTAT Calibrator).
- Prepare a serial dilution of the 1:11 diluted HEMOSTAT Calibrator [CAL] (Cal 1) using 0.9 % Sodium chloride [NaCl] to obtain the calibrator levels Cal 2, Cal 3 and Cal 4.

Example with a HEMOSTAT Calibrator [CAL] showing an analytical value of 88 % of norm:

Please note: Cal 1 level is diluted 1:11 – equivalent to pre-dilution of samples				
Preparation of Calibrator Dilutions				
	Cal 1	Cal 2	Cal 3	Cal 4
AT* % of norm	Cal 1 [%]	Cal 1 [%] / 2	Cal 1 [%] / 4	Cal 1 [%] / 8
Example AT % of norm	88.0 %	44.0 %	22.0 %	11.0 %
HEMOSTAT Calibrator	20 µl of [CAL]	100 µl of Cal 1	100 µl of Cal 2	100 µl of Cal 3
0.9 % NaCl	200 µl	100 µl	100 µl	100 µl

*LOT-specific analytical value of the calibrator. It can be found on the table of analytical values in the calibrator kit HEMOSTAT Calibrator ([REF] 35500).

- Measure the prepared calibrator levels in duplicates and write down or print the respective kinetic results [mE/min]. Calculate the mean value [mE/min] of each duplicate.
Please note: Ignore values for [%], as those are derived from a previous calibration.
- Insert the calculated mean values into the instrument by the following steps:


Choose the test **AT** by pressing the enter key  (the message “cuv(ette) in” appears).

Press the -key, enter the first data point ([%] from a) and press .

Enter result [mE/min] from c) and press .

Repeat this process until all calibration points are inserted.

Please note: the 5th calibration point must be 0.

- Add the LOT number of HEMOSTAT Antithrombin^{liquid} reagent and press  repeatedly to save new parameters

On-Board Stability

Material	Time [h]
[RGT] Antithrombin Reagent at 37°C	3
[SUB] Substrate at 37°C	6

The stated stability data were established under controlled laboratory conditions. The above mentioned on-board stability values may deviate due to differences in laboratory environmental conditions.

Test Settings

Test Hemostat AT	
<i>(Reduced Setup, User) <AT> + Enter-Key = CuvIN or Pat-ID + 0-key</i>	
Method Store	6
AT	
Date	Will be displayed
Measuring Time	65 s
Gain_idx	0
Cuv in	ON
Reag_sens	ON
Start Reagent	
LOT	Please insert LOT number
Volume	30 µl
incu	180 s
Clotting	OFF
Kin/ Dif	ON
Calibration	
3 rd conversion	INTERPOLATION
1. point: Insert LOT-specific calibration value Cal 1 [%]	Insert calculated mean value [mE/mn] for Cal 1
2. point: Insert LOT-specific calibration value Cal 2 [%]	Insert calculated mean value [mE/mn] for Cal 2
3. point: Insert LOT-specific calibration value Cal 3 [%]	Insert calculated mean value [mE/mn] for Cal 3
4. point: Insert LOT-specific calibration value Cal 4 [%]	Insert calculated mean value [mE/mn] for Cal 4
5. point: Leave empty: 0.0 [%]	Leave empty: 0.0 [mE/min]

Interference Studies

No interference up to ...					
Bilirubin	mg/dl	50	spiked normal plasma	50	spiked pathological plasma
Hemoglobin	mg/dl	1000	spiked normal plasma	1000	spiked pathological plasma
Lipids	mg/dl	1000	spiked normal plasma	1000	spiked pathological plasma

Performance Characteristics

Measuring interval	
Analytical measuring interval	11 % to 130 %

Reference Interval			
The following data was obtained with a specific HEMOSTAT Antithrombin ^{liquid} LOT using normal plasma according to EP28-A3.			
HumaClot Quattro	Median	95 % Reference interval	
		2.5th Percentile	97.5th Percentile
143 samples	95.3 %	74.3 %	129 %
Please note: The reference intervals vary from laboratory to laboratory depending on the population served, technique and reagent LOT used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the mentioned variables are changed.			
For more information how to establish reference intervals see CLSI document C28-A3.			

