

Application Sheet for Prothrombin Time (PT) with HEMOSTAT Thromboplastin-SI

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 Thromboplastin-SI	31002		
RGT Thromboplastin reagent		6 x 2 ml	Heated reagent position (HC Junior) Left heated reagent position with magnetic stirrer and reducer ring on HumaClot Duo Plus and Quattro
BUF Reconstitution medium		6 x 2 ml	for reconstitution of RGT
HEMOSTAT Thromboplastin-SI	31003		
RGT Thromboplastin reagent		6 x 10 ml	Left heated reagent position with magnetic stirrer on HumaClot Duo Plus and Quattro). Not applicable on HumaClot Junior.
BUF Reconstitution medium		6 x 10 ml	for reconstitution of RGT only
CAL HEMOSTAT Calibrator	35500	4 x 1 ml	-
NaCl 0.9 % Sodium chloride	-		for dilution of HEMOSTAT Calibrator
CPN HEMOSTAT Control Plasma Normal	35001	6 x 1 ml	-
CPA HEMOSTAT Control Plasma Abnormal	35002	6 x 1 ml	-
Cuvettes dispo incl. mixer	15660/10	5 x 100 pcs	Pre-heated cuvette positions
Cuvettes bag incl. mixer	15660/11	500 pcs	
Cuvettes bag incl. mixer	15660/12	5 x 500 pcs	
Reducer Ring	15660/52	2 pcs	Standard accessory HumaClot Duo Plus and HumaClot Quattro
Magnetic stirrer	15800/50	10 pcs	For HumaClot Duo Plus and Quattro. (to be cleaned with HumaClot Pro Wash Solution; REF 15800/20)

Pipetting Scheme

Pipetting Scheme	
Pre-warm [RGT] Thromboplastin reagent (reconstituted) and cuvettes at 37°C	
1. Sample	50µl
Transfer cuvette with sample to a measuring channel	
Incubation time	60 s
2. Start reagent [RGT] Thromboplastin reagent	100 µl
Auto start	yes

Standard Curve Calibration

A new standard curve needs to be established when

- changing to a new HEMOSTAT Thromboplastin-SI 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 PT %-value in the analytical value sheet of the HEMOSTAT Calibrator **[CAL]**.

- Calculate the respective % of activity-value (Quick) 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 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 92 % activity:

Preparation of Calibrator Dilutions				
	Cal 1	Cal 2	Cal 3	Cal 4
% PT* (% Quick)	Cal 1 [%]	Cal 1 [%] / 2	Cal 1 [%] / 4	Cal 1 [%] / 8
Example % PT*	92.0 %	46.0 %	23.0 %	11.5 %
HEMOSTAT Calibrator	400 µl	400 µl of Cal 1	400 µl of Cal 2	400 µl of Cal 3
0.9 % NaCl	0 µl	400 µl	400 µl	400 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 including Cal 1 in duplicates and write down or print the respective clotting time results [s]. Calculate the mean value [s] of each duplicate.
Please note: Ignore values for [%] and [INR], as those are derived from a previous calibration.
- Insert the calculated mean values into the instrument by the following steps:
Choose the test **PT** 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 [s] from c) and press .
Repeat this process until all calibration points are inserted.
Please note: the 5th calibration point must be 0.
- Add ISI and LOT number of **[RGT]** Thromboplastin reagent from the LOT-specific analytical value sheet and press  repeatedly to save new parameters.

Please note: the determination of an MNPT value is not needed when calibrating with the HEMOSTAT Calibrator.

On-Board Stability

Material	Time [h]
RGT Thromboplastin reagent (reconstituted)	16 (REF 31003 10 ml)
	8 (REF 31002 2 ml)

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 Protocol_ Printed automatically with every change / new start	
<i>(Reduced Setup, User) <1> + Enter-Key=CuvIN or Pat-ID + 0-Key</i>	
Method store	1
PT	
Date	Will be displayed
Meas.time	81 s
Gain_idx	0
Cuv in	ON
Reag_sens	OFF
Start Reagent	
LOT	Please insert LOT number
Volume	100 µl
Incubation	60 s
Clotting	ON
Kin/Dif	OFF
Calibration	
1 st conversion	INTERPOLATION
1. point: Insert LOT-specific calibration value Cal 1 [%]	Insert calculated mean value [s] of Cal 1
2. point: Insert LOT-specific calibration value Cal 2 [%]	Insert calculated mean value [s] of Cal 2
3. point: Insert LOT-specific calibration value Cal 3 [%]	Insert calculated mean value [s] of Cal 3
4. point: Insert LOT-specific calibration value Cal 4 [%]	Insert calculated mean value [s] of Cal 4
5. point: Leave empty: 0.0 [%]	Leave empty: 0.0 [s]
2 nd conversion	INR
ISI	Insert LOT- and instrument specific ISI
MNPT (=> 100%)	Will be displayed after calibration is saved

Please note:

1st conversion (→ %) and 2nd conversion (→ INR) are optional and can be switched off by entering “none” into the corresponding field.

If only the 1st conversion (→ %) is switched off, it is required to enter the MNPT value manually. For further information please refer to the User Manual.

Interference Studies

No interference up to ...					
Bilirubin	mg/dl	50	spiked normal plasma	37.5	spiked pathological plasma
Hemoglobin	mg/dl	674	spiked normal plasma	500	spiked pathological plasma
Lipids	mg/dl	1000	spiked normal plasma	364	spiked pathological plasma

Performance Characteristics

Measuring interval	
Valid Clotting	9 - 80 s

Reference Interval			
<p>The following data was obtained with a specific HEMOSTAT Thromboplastin-SI LOT using normal plasma according to EP28-A3.</p>			
HumaClot Quattro	Median	95 % Reference interval	
		2.5th Percentile	97.5th Percentile
170 samples	11.8 s	10.4 s	13.7 s
170 samples	89.4 %	67.4 %	114 %
170 samples	1.03 INR	0.87 INR	1.24 INR
<p><i>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.</i></p>			
<p>For more information how to establish reference intervals see CLSI document C28-A3.</p>			

