Application Sheet for free protein S with HEMOSTAT free Protein S

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/36201.pdf
www.human-de.com/data/gb/vr/36201.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 free Protein S	36201		
RGT free PS Latex Reagent		2 x 2.5 ml	Beside the analyzer
BUF Reaction Buffer		2 x 4 ml	Beside the analyzer
DIL Diluent		2 x 6.5 ml	For mandatory sample dilution
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	-
Cuvettes with pre-filled mixers	15660/10	5 x 100 pcs	
Cuvette bag with separate mixer	15660/11	500 pcs	Pre-heated cuvette positions
Cuvette bag with separate mixer	15660/12	5 x 500 pcs	

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Pipetting Scheme

Please measure pre-diluted sample within 15 min, to avoid sample degradation and false too high results.

Sample Pre-Dilution (1:6)	
Sample, Control	25 µl
DIL free Protein S Diluent	125 µl
Pipetting	
Prewarm cuvettes at 37°C	
1. BUF Reaction buffer	55 µl
2. Pre-diluted sample	25 µl
Transfer cuvette with pre-diluted sample and reaction buffer into the measuring channel	
Incubation time	120 s
3. Start Reagent RGT free PS Latex Reagent	**70 µl**
Auto start	yes

Standard Curve Calibration

A new standard curve must be established when
- changing to a new HEMOSTAT free Protein S 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 fPS %-value in the analytical value sheet of the HEMOSTAT Calibrator CAL.
a) For free Protein S a 5-point calibration with fixed calibrator points needs to be performed:
   [%] fPS-values 10 %, 30 %, 60 %. 90% and 120 %.
b) Calculate required volumes of the Diluent DIL and prepare the dilution levels of the calibrator:

Example with a HEMOSTAT calibrator showing an analytical value (**AV**) of 92% activity:

Preparation of dilutions			
	[%] free PS	**Volume Diluent DIL [µl]**	**Volume Calibrator CAL [µl]**
Cal 1	10	= AV*6 − 10 µl = 92·6 − **10** = 542	**10**
Cal 2	30	= AV*6 − 30 µl = 92·6 − **30** = 522	**30**
Cal 3	60	= AV*6 − 60 µl = 92·6 − **60** = 492	**60**
Cal 4	90	= AV*6 − 90 µl = 92·6 − **90** = 462	**90**
Cal 5	120	= AV*6 − 120 µl = 92·6 − **120** = 432	**120**

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).

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

   Choose the test *fPS* 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 6th calibration point must be 0.

e) Add LOT number of HEMOSTAT free Protein S reagent and press [↵] repeatedly to save new parameters.

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On-Board Stability

Material	Time [h]
RGT free PS Latex Reagent	68
BUF Reaction Buffer	68
DIL Diluent	68

The stated stability data were established under controlled laboratory conditions (18 – 25°C). The above-mentioned stability values may deviate due to differences in laboratory environmental conditions.

To optimize the stability in applying HEMOSTAT free Protein S the following handling is recommended:
- Reagents in original vials may be continually at 18 – 25°C for 68 hours.
- When used temporally at 18 – 25°C, the stability can be extended for max. 3 weeks:
  - tests have to be performed 2 days per week for max. 2 hours at 18 – 25°C with an in-between storage at 2 – 8°C.

Test Protocol_Printed automatically with every change / new start	
(Reduced Setup, User) <8>+Enter-Key=cuv in or Pat-ID + 0-Key	
Method store	8
fPS	
Date:	Will be displayed
Measuring Time	111 s
Gain_idx	0
Cuv in	ON
reag_sens	OFF
reag_mode	0
clot_mode	0
Start Reagent:	
Lot	**Please insert LOT number**
Volume	70 µl
Incubation	120 s
Clotting	OFF
Kinetic	ON
Calibration	
3rd conversion	INTERPOLATION
1. point: 10.0 [%]	**Insert calculated mean value [mE/mn] for Cal 1**
2. point: 30.0 [%]	**Insert calculated mean value [mE/mn] for Cal 2**
3. point: 60.0 [%]	**Insert calculated mean value [mE/mn] for Cal 3**
4. point: 90.0 [%]	**Insert calculated mean value [mE/mn] for Cal 4**
5. point: 120.0 [%]	**Insert calculated mean value [mE/mn] for Cal 5**
6. point: 0.0 [%]	**Leave empty:** 0.0 [mE/mn]

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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	500	spiked normal plasma	500	spiked pathological plasma

Performance Characteristics

Measuring Interval	
Analytical measuring interval	10 % to 130 %
Reportable interval	1.7 % to 260 %

The analytical measuring interval of HEMOSTAT free Protein S covers 10 % to 130 %.

The reportable interval can be extended by diluting a sample displayed as > 130 %

- Re-Run the sample with further 1:2 dilution (total sample dilution = 1:12) with HEMOSTAT free Protein S Diluent DIL and measure immediately. The resulting value needs to be multiplied by 2 to obtain the true value.

Samples below the measuring interval will be displayed as < 10 % or as "ERR lin".

- In this case the patient's sample can be measured undiluted and result needs to be divided by 6 to calculate true free Protein S value.

Reference Interval

The following data was obtained with a specific HEMOSTAT free Protein S LOT using normal plasma according to EP28-A3.

HumaClot Quattro	Median	95% Reference interval	
		2.5th Percentile	97.5th Percentile
88 females	89.7 %	47.4 %	149 %
104 males	113 %	81.2 %	162 %

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.

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