Human Haptoglobin Kit for use on SPAPLUS®

For *in vitro* diagnostic use only

Product Code: NK058.S

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FDA (USA) Information
Analyte name: Haptoglobin
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is intended for the quantitative *in vitro* measurement of human haptoglobin in serum, lithium heparin or EDTA plasma, using the SPAPLUS turbidimetric analyser. Measurement of haptoglobin is an aid in the diagnosis of haemolytic diseases related to the formation of haemoglobin-haptoglobin complexes. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

Haptoglobin is an acid α2 acute-phase plasma glycoprotein and binds specifically to free plasma oxy-haemoglobin. The high molecular weight complex thus formed prevents filtering of haemoglobin by the kidneys[1]. Low levels of haptoglobin are associated with haemolytic anaemias[2] and liver disease [3,4]. Very high levels can develop in response to inflammatory conditions[5,6]

3 PRINCIPLE

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 **Human haptoglobin antiserum:** This antiserum is monospecific for haptoglobin and is supplied in stabilised liquid form. It contains 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 0.1% EDTA and 0.01% benzamidine as preservatives

4.2 **Calibrator and controls:** These consist of pooled human serum and are supplied in stabilised liquid form. The concentration of haptoglobin given on the quality control certificate has been obtained by comparison with the DA470k international reference material. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.3 **Reaction buffer:** Containing 0.099% sodium azide as a preservative.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either cleared by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The Human haptoglobin antiserum, Reaction buffer, calibrators, and controls may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human haptoglobin antiserum and Reaction buffer may be stored, uncapped, on the SPAPLUS analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum, lithium heparinised or EDTA plasma samples. Samples should be obtained by venepuncture and in the case of plasma separated as soon as possible. Blood should be allowed to clot and the serum separated as soon as possible to prevent haemolysis. Samples may be stored at 2-8°C for up to 7 days, but for prolonged storage samples should be kept frozen at -20°C or below. Repeated freeze/thaw cycles should be avoided. Microbially contaminated serum samples, samples containing particulate matter and lipaemic or haemolysed serum samples should not be used.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Human Haptoglobin Antiserum SPAPLUS*
8.1.2 1 x *Human Haptoglobin SPAPLUS Calibrator 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human Haptoglobin SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human Haptoglobin SPAPLUS Low Control*
8.1.5 1 x 100 Tests *Haptoglobin Reaction Buffer SPAPLUS*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.

For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 37.

Item Name 37 HAPT
DATA INFORMATION
Units g/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 340 ▼
Sub W.Length ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION Auto Fill
Type Logit 2 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

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Item Name 37 HAPT

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 10
REAGENT1 VOL 165 µL
REAGENT2 VOL 50

Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**

DATA PROCESS
READ **ABSORBANCE LIMIT**

	START	END	
MAIN	53	54	LOW -3.0
SUB	30	31	HIGH 3.0

FACTOR **Reaction Check**
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2.0 CHECK POINT
LINEAR CHECK (%) 0 LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High 20 ▼
Auto Rerun Dilution Rate Low ▼

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

PROZONE CHECK

	START	END	LIMIT (%)	Min dOD	
FIRST	[ ]	[ ]			
SECOND	[ ]	[ ]	[ ]	○ Low	● High
THIRD	[ ]	[ ]	[ ]	○ Low	● High

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*Automatically calculated

Item Name 37 HAPT

Auto Rerun SW
● On ○ Off
Auto Rerun Range (Result)
● On ○ Off ● On ○ Off
Lower Higher

	Lower	Higher
Serum	Cal 1 #	Cal 6 #
Urine		
Plasma		
CSF		
Dialysis		
Other		

Bottle Size (ml)

24 Items		36 Items
Reagent1	60	Reagent1
Reagent2 R1	17.5	Reagent2 R1
Reagent2 R2	6	Reagent2 R2

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ● On ○ Off
Higher ● On ○ Off

Prozone Range ○ On ● Off

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The calibrator (Standard #) values are found in the Quality Control Certificate (SIN209.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing the Autofill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter pages 3 and 4 to ensure correct value entry.

*****The Blank correction factor is automatically calculated by the instrument.

8.5 Measuring range

The approximate measuring range of the haptoglobin assay is shown in the table below.

SPA PLUS analyser dilution	Approximate range (g/L)
1/1 (neat)	0.026-0.4
1/10	**0.26-4.0**
1/20	0.52-8.0

9 QUALITY CONTROL

9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPA PLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to the local technical support organisation.

9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN209.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of highly lipaemic or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 This assay has not been validated using paediatric samples.

10.3 Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of Haptoglobin measurements alone. Clinical history and other laboratory findings must be taken into account.

11 EXPECTED VALUES

The reference range for this kit was transferred from an alternative commercially available assay in accordance with CLSI document *EP C28-A3 "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory"* and was validated by measuring the haptoglobin concentration of sera taken from 20 healthy UK adults. Wherever possible it is strongly recommended that local ranges are generated.

	95 Percentile Range (g/L)
Haptoglobin	0.32 - 1.97

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2)**.** The study was performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers.

Haptoglobin precision summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	3.415	0.070	2.1%	0.089	2.6%	0.060	1.8%	0.128	3.7%
Serum 2	1.945	0.026	1.3%	0.037	1.9%	0.036	1.8%	0.058	3.0%
Serum 3	0.470	0.008	1.7%	0.011	2.4%	0.022	4.7%	0.026	5.5%

12.2 Comparison

A correlation study was performed on 78 samples (30 normal sera and 48 clinical sera) using this kit on a SPA PLUS and an alternative commercially available Haptoglobin assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 0.97x + 0.03 (g/L) (y = SPA PLUS Haptoglobin; x = alternative assay)

correlation coefficient r = 0.9925 (calculated by linear regression)

12.3 Limit of Quantitation

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* the limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 0.026g/L based upon the minimum sample dilution.

12.4 Linearity

A linearity study was performed following CLSI (formally NCCLS) *Evaluation of the Linearity of Quantitative Measurement Procedures* document EP6-A. One user assessed the linearity of a pool of high samples using one lot of reagent on one analyser. This gave a regression plot of y = 0.974x +0.043g/L R^2 = 0.996 (y = measured Haptoglobin concentration, x = theoretical concentration) over the range of 0.34-3.87g/L.

12.5 Interference

No significant assay interference using 1500 formazine turbidity units (FTU) of chyle and 200mg/L bilirubin, has been demonstrated at the minimum sample dilution (1/1).

	Bilirubin	Chyle
Mean Haptoglobin (g/L)	0.27	0.25
% interference	0.38%	-5.31%

Haemoglobin interference: As haptoglobin specifically binds free haemoglobin to form the haemoglobin-haptoglobin complex, haemoglobin interference testing is not suitable for this assay.

12.6 Antigen excess

No antigen excess was observed to a level of approximately 8g/L.

13 BIBLIOGRAPHY

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