

Haptoglobin

For the Quantitative Determination of Human Haptoglobin in Serum

Cat. No. KAI-022

INTENDED USE

For the quantitative determination of human haptoglobin in human serum by immunoturbidimetric assay. Haptoglobin is a protein that binds hemoglobin, the oxygen-carrying pigment in red blood cells. Measurement of haptoglobin may aid in the diagnosis of hemolytic diseases (diseases in which the red blood cells rupture and release hemoglobin) relating to the formation of hemoglobin-haptoglobin complexes and certain kidney diseases. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

The **K-ASSAY**® Haptoglobin assay is intended for the quantitative determination of human haptoglobin by immunoturbidimetric assay. The antiserum used in the kit was produced against purified human haptoglobin. The haptoglobin antibody interacts with the haptoglobin in the serum forming immune complexes. The immune complexes cause an increase in light scattering which correlates with the concentration of serum haptoglobin.^{4,5}

Haptoglobin is a serum protein which binds hemoglobin, the oxygen-carrying pigment in red blood cells. Measurement of haptoglobin may aid in the diagnosis of hemolytic diseases related to the formation of hemoglobin-haptoglobin complexes and certain kidney diseases. Haptoglobin, an acute phase protein, is a potential reporter molecule for glycosylation changes in the serum which may increase in inflammation and liver disease.¹

Haptoglobin has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay.^{2,3} The **K-ASSAY**® Haptoglobin assay uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The **K-ASSAY**® Haptoglobin assay quantifies the haptoglobin in the patient's serum based on immunoturbidimetric assay.

Calibrators, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and antibody diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antiserum is added to the cuvettes. The samples (antigen) and antiserum are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering which correlates with the concentration of serum haptoglobin. Following an incubation period lasting approximately 5 minutes, the

absorbance of the solution is measured at 600 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples are interpolated from the calibration curve. The antiserum used in the kit is a goat polyclonal antibody specific for human haptoglobin.

The **K-ASSAY**® Haptoglobin assay is suitable for two-reagent clinical chemistry autoanalyzers that use multi-point calibration. The assay is run using the **K-ASSAY**® Multi-Analyte Calibrator. Six calibrators are used to prepare a standard curve for quantifying the levels of haptoglobin present in the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent	3 x 18 mL
Tris(hydroxymethyl)aminomethane	
R2: Antiserum Reagent	2 x 7 mL
Anti-human haptoglobin goat antiserum (50%)	

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Guide Management No. CDC-22 issued by the Center for Disease Control, Atlanta, Georgia.

WARNING: This product can expose you to chemicals including thiourea which is known to the State of California to cause cancer/birth defects or other

reproductive harm. For more information go to www.P65Warnings.ca.gov. The R-1 reagent contains 0.009% of thiourea (CAS No. 62-56-6).

REAGENT PREPARATION

The reagents are ready to use. They do not need to be reconstituted.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Opened reagents can be used for one month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 600 nm. Refer to the instrument manual from the manufacturer regarding the following:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics, operating instructions
- Calibration procedures including materials and / or equipment to be used
- Operational precautions, limitations, and hazards
- Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass) within 2 hours. Serum should be stored refrigerated (2-8°C) and can be used within one week or should be stored frozen for up to 2 months.

Use plastic tubes for storing the sample, do not use glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent	3 x 18 mL
Reagent 2 (R-2) Antiserum Reagent	2 x 7 mL

Materials Required But Not Supplied

Calibrators: **K-ASSAY**® Multi-Analyte Calibrator, Cat. No. KAI-016C. (6 calibrators containing human serum with known levels of haptoglobin).

Two Reagent Clinical Chemistry Analyzer:
 Capable of accurate absorbance readings at 600 nm
 Capable of accurately dispensing the required volumes
 Capable of maintaining 37°C

Assay Procedure

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717):

Sample	3 µL
↓	
• ← R1 (Buffer Reagent)	250 µL
↓	37 °C, 5 min.
• ← R2 (Antiserum Reagent)	70 µL
↓	37 °C, 5 min.
2-point endpoint, 600 nm	

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37°C
TEST	(HP)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(3) ()
R-1 VOLUME	(250) () (NO)
R-2 VOLUME	(70) () (NO)
WAVELENGTH	() (600)
CALIB. METHOD	(NONLINEAR) (4) (6)
STD.(1) Conc.-POS.	(* 1) - (1)
STD.(2) Conc.-POS.	(* 2) - (2)
STD.(3) Conc.-POS.	(* 3) - (3)
STD.(4) Conc.-POS.	(* 4) - (4)
STD.(5) Conc.-POS.	(* 5) - (5)
STD.(6) Conc.-POS.	(* 6) - (6)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-320000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.00)

*1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that haptoglobin levels be determined using a multi-point calibration curve prepared using the **K-ASSAY**® Multi-Analyte Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and number / type of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the stated recovery range.

LIMITATIONS OF PROCEDURE

The measurable range for haptoglobin is between 20 to 220 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

If the haptoglobin concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensate for the dilution.

PERFORMANCE

Precision

The precision for the **K-ASSAY**® Haptoglobin assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

Precision Assay: Within Run

<u>Sample I</u>	<u>Sample II</u>
N = 20	N = 20
Mean = 68.15	Mean = 366
SD = 0.88	SD = 5.5
CV = 1.3%	CV = 1.5%

Precision Assay: Between Runs

<u>Sample I</u>	<u>Sample II</u>
N = 8	N = 8
Mean = 85.9	Mean = 225.6
SD = 0.8	SD = 1.4
CV = 0.972%	CV = 0.624%

Accuracy / Correlation

A comparison of the **K-ASSAY**® Haptoglobin assay and an Incstar Haptoglobin Test Kit was performed using a Hitachi 717. The test results provided the following data:

$$\begin{aligned}y &= 0.937x - 9.25 \\r &= 0.993 \\n &= 60 \\x &= \text{Incstar Haptoglobin Test Kit} \\y &= \text{K-ASSAY}^{\circledR} \text{ Haptoglobin assay}\end{aligned}$$

x min = 34	y min = 4
max = 266	max = 267
mean = 109	mean = 93

Assay Range

20 - 220 mg/dL (multi-point calibration)

INTERFERENCE

Bilirubin C	No interference up to 20 mg/dL
Bilirubin F	No interference up to 20 mg/dL
Intralipid	No interference up to 500 mg/dL

Haptoglobin binds tightly to free hemoglobin. Use of hemolyzed samples is not recommended as will have a negative effect on this assay's results.

EXPECTED VALUE

The expected value as reported is between 30 to 178 mg/dL. Each laboratory should establish its own expected values using this kit.

REFERENCES

1. Turner, G.A. "Haptoglobin, a Potential Reporter Molecule for Glycosylation Changes in Disease." *Adv. Exp. Med. Biol.* 376: 231-238, 1995.
2. Bergstrom, K., *et al.* "An Automated Turbidimetric Immunoassay for Plasma Proteins." *Scand. J. Clin. Lab. Invest.*, 40:637, 1980.
3. Heidelberger, M. and F. Kendall. *J. Exp. Med.*, 61: 563, 1935.
4. Killingsworth, L.M. and Savory, J., "Nephelometric Studies on the Precipitin Reactions," *J. Clin. Chem.*, 19: 403-407, 1973.
5. Sternberg, J.C. "A Rate Nephelometer for Measuring Specific Proteins by Immunoprecipitin Reaction," *Clin. Chem.*, 23:1456-64, 1977.
6. Lizana, J. and Hellina, K., "Manual Immunonephelometric Assay of Proteins with Use of Polymer Enhancement." *Clin. Chem.* 20:1181, 1974.

LABELING SYMBOLS

 LOT	Lot Number
 RGT	Reagent
	Expiration or "Use By" Date
 REF	Catalog Number
 IVD	For <i>In Vitro</i> Diagnostics Use
 2-8 °C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Consult Package Insert for Instructions for Use
 EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE



 **EC REP**

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