HEME Assay Kit

Cat. No. Heme-018kit **Lot. No.** (See product label)

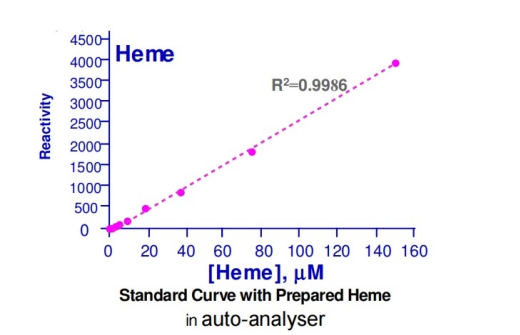
SPECIFICATION

Product Overview	Colorimetric Determination of Total Heme at 670 nm using Auto-Analyser
Description	Heme is a complex molecule with an iron atom at its center, surrounded by a porphyrin ring. This structure enables heme to bind with oxygen and other molecules, making it essential for oxygen transport and carbon dioxide removal in the blood. In medical examinations, heme is crucial for several tests. Measuring hemoglobin levels assesses the blood's oxygen-carrying capacity. The presence of heme in urine can indicate conditions such as hemolytic anemia, renal diseases, and certain cancers.
Kit Compoents	Kits suitable for performing 200 assays in auto-analyser format are available from our company. The kits contain the full assay method plus: Bottle 1: Regent1 (Apo-HRP plus Buffer): 40 mL, pH=6.5±0.1. Stable for> 1 year at 4 centigrade. Bottle 2: Regent2 (color agent): 10ml, pH=3.33. Stable for> 1 year at 4 centigrade. Bottle 3: Calibrator (equivalent to 62.5 μM heme): 2 mL. Stable for> 1 year at 4 centigrade.
Principle	Horseradish Peroxidase (HRP) is a glycoprotein enzyme extracted from the roots of the horseradish plant (Armoracia rusticana). When it attaches to a Heme group, it is able to catalyze the oxidation reaction by H2O2. In this assay, HRP will catalyze a oxidation reaction, which transform a color agent into a blueish complex. It can be detected by spectrophotometer at 670nm, which can be used to calculate the concentration of Heme groups in the sample.
Calculation	Subtract blank OD (water) from the Calibrator and Sample OD values. The total heme concentration of sample is calculated as: 62.5 × n (μM)× (OD: SAMPLE OD:

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	BLANK)/ (OD: CALIBRATOR - OD: BLANK)) OD: SAMPLE, OD: CALIBRATOR and OD: BLANK are OD values of the sample, the Calibrator and water. n is the dilution factor (e.g. 100 for blood samples diluted 100-fold in water). Conversions: 1mg/dL heme equals 15.3 µM, 0.001% or 10 ppm.
Usage	Notes: Serum and plasma samples can be assayed directly. Blood samples should be diluted 100-fold in distilled water. Reagent preparation: R1: solution 1 (Apo-HRP plus Buffer): 200 µL Sample, Calibrator and blank (water): 10 µL R2: solution 2 (color agent plus additives): 50 µL Incubate 5 minutes. Detected at 670 nm. The prepared reagent is stable for more than 2 days when refrigerated. Read OD of Sample, Calibrator, Blank from the auto-analyser in above condition.
Applications	Direct Assays: Total heme in blood, serum, plasma, urine, heme-carrying enzymes. Pharmacology: Effects of drugs on heme metabolism. Drug Discovery: HTS for drugs that modulate heme levels.
Reference	1. Vijith Vijayan, Robert Greite, et al (2022). Determination of free heme in stored red blood cells with an apo-horseradish peroxidasebased assay. Biological Chemistry, 403 (11-12), 1091-1098. 2. Dalko, E., Tchitchek, N., Pays, L., Herbert, F., Cazenave, P. A., Ravindran, B. & Pied, S. (2016). Erythropoietin levels increase during cerebral malaria and correlate with heme, interleukin-10 and tumor necrosis factor-alpha in India. PloS one, 11 (7), e0158420. 3. Dos Santos, L. I., et al (2021). Disrupted iron metabolism and mortality during co-infection with malaria and an intestinal Gramnegative Extracellular pathogen. Cell Reports, 34 (2), 108613.

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Standard Curve	 Standard Curve with Prepared Heme in auto-analyser Heme was determined using a auto-analyser. For examination, a 150 µM Heme solution were made, then other 8 portions of solutions were made under the condition, that each concentration of the next solution is half of the last one. (the last portion's concentration should be around 0.5859 µM)

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