# Heme Detection Kit (ELISA)

**Cat. No.** KITG-038 **Lot. No.** (See product label)

## SPECIFICATION

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| **Product Overview** | The Heme Detection Kit (ELISA) is a sandwich enzyme-linked immunosorbent assay kit for the quantitative detection of heme concentration in serum, plasma, and other biological samples. This kit is intended for research use only. |
| **Species** | Human |
| **Description** | The kit employs a double-antibody sandwich ELISA method. A capture antibody is pre-coated onto the microplate. The sample is added, and the antigen in the sample is captured by the coated antibody. Subsequently, an HRP-labeled detection antibody is added, forming an 'antibody-antigen-enzyme-labeled antibody' sandwich complex. After washing to remove unbound substances, TMB substrate is added for color development. Stop solution is then added, and the optical density (OD) is measured at 450 nm using a microplate reader. The color intensity is directly proportional to the concentration of heme in the sample. |
| **Form** | ELISA Kit |
| **Bio-activity** | Linear range: 2.5-160 pg/mL Sensitivity: 25 pg/mL Intra-assay precision: CV < 10% (N = 20) Inter-assay precision: CV < 15% (N = 20) Analytical specificity: No significant cross-reactivity observed with other related factors at 10 μg/mL in dilution buffer |
| **Applications** | Quantitative detection of heme concentration in serum, plasma, tissue homogenates, cell lysates, and cell culture supernatants |
| **Notes** | Quality Control Test: Each batch of the kit has been verified for performance using sta |

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ndard quality control procedures. Sample Collection and Storage: Serum: Collect whole blood using serum collection tubes. Allow blood to clot at room temperature, then centrifuge at 1000×g for 15 minutes to obtain serum. Handle gently to avoid hemolysis. If testing cannot be performed immediately, aliquot the serum and store at -20°C or below for up to 6 months. Samples should not be freeze-thawed more than 2 times. Plasma: Collect whole blood using EDTA or heparin anticoagulant tubes. Centrifuge at 1000×g for 15 minutes to obtain plasma. Handle gently to avoid hemolysis. If testing cannot be performed immediately, aliquot the plasma and store at -20°C or below for up to 6 months. Samples should not be freeze-thawed more than 2 times. Tissue: Weigh and mince tissue. Add lysis buffer at a ratio of 1:4 to 1:9 (w/v), for example, 100 mg tissue in 400 µL lysis buffer. Adjust the ratio as needed for the experiment. Homogenize and centrifuge at 5000×g for 5-10 minutes. Use the supernatant for detection. Cells: Take an appropriate amount of cells, sonicate on ice, then centrifuge at 5000×g for 5-10 minutes. Use the supernatant for detection. Cell culture supernatant: Centrifuge cell culture supernatant at 5000×g for 5-10 minutes. Use the supernatant for detection. Stability: Serum and plasma samples can be stored at -20°C or below for up to 6 months. Samples should not be freeze-thawed more than 2 times. Technical Notes: It is recommended to perform a pilot experiment to determine the optimal dilution factor for samples. When sample concentration exceeds the upper limit of the detection range, samples should be appropriately diluted and re-tested. The calculated result should be multiplied by the dilution factor. When sample concentration is below the limit of quantification (LOQ), the detection result cannot be accurately quantified. Do not use components from different batches of the kit together. Do not assemble microplate strips from different plates for detection, even if they are from the same batch, as plate-to-plate variations may exist. A standard curve must be freshly prepared for each experiment. Do not use a previous standard curve for calculating results of a new experiment. Do not use the kit beyond the expiration date. The substrate chromogen should be colorless. If it turns blue, it has deteriorated and should not be used. Use appropriate sealing film to seal the microplate wells for more reliable results. Avoid bubble forma

tion during pipetting. Do not cross-use caps from different reagent bottles. Follow the protocol strictly. Any deviation from the protocol may result in altered results. For any non-standard experimental procedures, consult technical support in advance. Otherwise, the reliability of the results cannot be guaranteed. Warning: The stop solution contains dilute sulfuric acid and is corrosive; handle with care. The chromogen, concentrated wash buffer may cause skin, eye, and respiratory irritation. Wear a mask to avoid inhaling mist. Wear protective gloves, clothing, eye and face protection. Wash hands thoroughly after handling. The kit components contain preservatives and protein components that may cause allergic skin reactions. Scientific Background: Heme is an iron-containing porphyrin that serves as a prosthetic group for hemoglobin, myoglobin, and various enzymes including cytochromes and catalases. It is essential for oxygen transport, electron transfer, and catalytic reactions. Quantitative measurement of heme levels is important for understanding heme metabolism, iron homeostasis, and related pathological conditions. Features & Benefits: High sensitivity and specificity Wide linear detection range (2.5-160 pg/mL) Pre-coated microplate for convenience Compatible with multiple sample types Quantitative results with standard curve Target Species: Human Detection Method: Sandwich ELISA (Double-antibody sandwich method) Compatible Sample Types: Serum, plasma, tissue homogenates, cell lysates, cell culture supernatants Size: 48 Tests / 96 Tests Kit Components: Microplate (Pre-coated with capture antibody): 8 × 6 strips (48T) / 8 × 12 strips (96T) Calibrator (10×): Analyte at 1600 pg/mL - 1 vial × 100 µL (48T) / 1 vial × 200 µL (96T) Enzyme Conjugate (100×): 100× concentrated HRP-labeled detection antibody - 1 vial × 50 µL (48T) / 1 vial × 100 µL (96T) Universal Diluent (20×): Sample/calibrator/enzyme conjugate universal diluent - 1 vial × 10 mL (48T) / 1 vial × 15 mL (96T) Concentrated Wash Buffer (20×): 20× concentrated wash buffer - 1 vial × 15 mL (48T) / 1 vial × 25 mL (96T) Chromogen: TMB and hydrogen peroxide - 1 vial × 6 mL (48T) / 1 vial × 12 mL (96T) Stop Solution: Dilute sulfuric acid - 1 vial × 3 mL (48T) / 1 vial × 6 mL (96T) Materials Required but Not Supplied: Distilled or deionized water Disposable centrifuge tubes, disposable gloves, and other consumables Pipettes, multichannel pipettes, and compatible tips Beak

ers, graduated cylinders, reagent bottles, and other containers Sealing film or equival ent for sealing microplate wells Microplate shaker (if needed), centrifuge, vortex mixe r, and other auxiliary equipment Incubator, water bath, microplate reader, and plate w asher Preparation: 1× Wash Buffer preparation: If crystals precipitate in the concentra ted wash buffer (20×), heat at 37°C until all crystals are completely dissolved. Dilute 1:20 with distilled water (for example, 10 mL concentrated wash buffer + 190 mL distill ed water). Mix well. 1× Universal Diluent preparation: Dilute 1:20 with distilled water (f or example, 10 mL 20× concentrated universal diluent + 190 mL distilled water). Mix well. 1× Enzyme Conjugate Working Solution preparation: Dilute the 100× enzyme co njugate 1:100 with 1× universal diluent (for example, 10 µL 100× enzyme conjugate + 990 µL 1× universal diluent). Mix well. The prepared 1× enzyme conjugate working so lution can be stored at 2-8°C for up to 8 hours. Working Calibrator preparation: Centrif uge the 10× calibrator for 30 seconds before opening to ensure all calibrator is conce ntrated at the bottom. Prepare 7 centrifuge tubes. Dilute the 10× calibrator 10-fold wit h 1× universal diluent to prepare calibrator S1 (for example, 50 µL 10× calibrator + 45 0 µL 1× universal diluent) to obtain 500 µL of S1. Add 250 µL of 1× universal diluent t o each of the subsequent 6 tubes (S2-S7). Perform serial 2-fold dilutions by transferri ng 250 µL from S1 to S2, then 250 µL from S2 to S3, and so on through S7. This crea tes 7 calibrator concentrations (S1, S2, S3, S4, S5, S6, S7). Use 1× universal diluent as blank calibrator S0. Assay Protocol: 1. Bring all reagents to room temperature and equilibrate for approximately 30 minutes before use. Alternatively, place reagents in a 37°C incubator for rapid equilibration to room temperature. Do not open the microplat e before it has returned to room temperature. 2. Arrange the plate layout: Setup calibr ator wells, sample wells, and blank wells. - Calibrator wells: Add 100 µL of each calibr ator (S1-S7, 7 concentrations) per well - Sample wells: Add 50 µL of 1× universal dilu ent, then add 50 µL of sample - Blank wells: Add 100 µL of S0 (1× universal diluent). 3. Incubation: Seal the plate with sealing film and incubate at 37°C for 60 minutes. 4. Wash: Wash the plate 3 times. After the final wash, invert the plate and tap on clean a bsorbent paper to remove residual liquid. 5. Add Enzyme Conjugate: Add 100 µL of

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| | 1× enzyme conjugate working solution to calibrator wells and sample wells. Add nothing to blank wells. 6. Incubation: Seal the plate and incubate at 37°C for 60 minutes. 7. Wash: Wash the plate 5 times. Remove residual liquid as described above. 8. Color Development: Add 100 µL of chromogen to each well. Incubate at 37°C protected from light for 15 minutes. 9. Stop Reaction: Add 50 µL of stop solution to each well. 10. Measurement: Measure absorbance at 450 nm (single wavelength) or 450/630 nm (dual wavelength) using a microplate reader. Analysis: Dual-wavelength results do not require blank subtraction and can be directly used for standard curve fitting and result calculation. The following curve fitting methods can be used. Select the fitting method with the best r value for result calculation: • Four-parameter logistic (4-P) curve fitting: Concentration values on the x-axis, absorbance values on the y-axis. • Polynomial curve fitting: 2nd order polynomial, 3rd order polynomial. • Double-logarithmic linear fitting: Take the logarithm of concentration values and the logarithm of absorbance values, then perform linear fitting. • Point-to-point fitting: Perform linear fitting between each pair of adjacent points separately, and calculate segmentally. It is recommended to use professional software for result fitting and calculation, such as ELISACalc, SPSS, GraphPad Prism, or equivalent. The final sample concentration should be multiplied by the sample dilution factor. Sensitivity: 25 pg/mL |
| **Storage** | Store the kit at 2-8°C. The prepared 1× enzyme conjugate working solution can be stored at 2-8°C for up to 8 hours. |
| **Shipping** | Shipped at ambient temperature. |
| **Usage** | For research use only. Not for diagnostic use. |

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