Datasheet for ABIN6720463

Haptoglobin CLIA Kit

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Overview

Quantity:	96 tests
Target:	Haptoglobin (HP)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.37 ng/M - 1000 ng/M
Minimum Detection Limit:	1.37 ng/M
Application:	ELISA

Product Details

Purpose:	The Chemiluminescent Immunoassay kit is designed for the in vitro sensitive quantitative measurement of haptoglobin in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates. We offer **validation data** (WB) **for the kit components**. So you can be sure to order a reliable ELISA kit product composed of high quality reagents.
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Specificity:	This assay has high sensitivity and excellent specificity for detection of Haptoglobin (Hpt)
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Haptoglobin (Hpt) and analogues was observed.

Product Details

Sensitivity:	0.53 ng/mL
Components:	• Pre-coated, ready to use black 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent • Detection Reagent A • Detection Reagent B • Assay Diluent A • Assay Diluent B • Reagent Diluent (if Detection Reagent is lyophilized) • Substrate A • Substrate B • Wash Buffer (30 x concentrate) • Instruction manual
Material not included:	1. Luminometer capable of reading 96-well microplates with the following parameters: lag time 30.0 secs, read time 1.0 sec/well . 2. Precision single or multi-channel pipettes and pipette tips with disposable tips. 3. Eppendorf Tubes for diluting samples. 4. Deionized or distilled water. 5. Absorbent paper for blotting the microtiter plate. 6. Container for Wash Solution

Target Details

Target:	Haptoglobin (HP)
Alternative Name:	Haptoglobin (Hpt) (HP Products)
UniProt:	P00738
Pathways:	Transition Metal Ion Homeostasis

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time.

	• Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. • Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. • There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed. • Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. • Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. • Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. • Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.
Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	3 h
Plate:	Pre-coated

Application Details

Protocol:	1. Prepare all reagents, samples and standards, 2. Add 100µL standard or sample to each well. Incubate 1 hours at 37 °C, 3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C, 4. Aspirate and wash 3 times, 5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C, 6. Aspirate and wash 5 times, 7. Add 100µL Substrate Solution. Incubate 10 minutes at 37 °C, 8. Read RLU value immediately.
Reagent Preparation:	1. Bring all kit components and samples to room temperature (18-25 °C) before use. 2. Standard - Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1,000 ng/mL. Prepare 7 tubes containing 0.6 mL Standard Diluent and produce a triple dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1,000 ng/mL, 333.33 ng/mL, 111.11 ng/mL, 37.04 ng/mL, 12.35 ng/mL, 4.12 ng/mL, 1.37 ng/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0 ng/mL. 3. Detection Reagent A and Detection Reagent B - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with Assay Diluent A and B, respectively (1:100). 4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x). 5. Substrate working Solution - Mix the substrate A and B by the ratio of 99:1 to make the substrate working solution. Mix thoroughly. For example, prepare 1,000µL Substrate working Solution with 990µL Substrate A + 10µL Substrate B. Note: 1. Making serial dilution in the wells directly is not permitted. 2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly. 3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting. 4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once. 5. Prepare Substrate working Solution within 15 minutes before assay. 6. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved. 7. Contaminated water or container for reagent preparation will influence the detection result.
Assay Procedure:	1. Determine wells for diluted standard, blank and sample. Prepare wells for standard points and blank. Add 100µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. Cover with the Plate sealer. Incubate for 2

hours at 37 °C.
2. Remove the liquid of each well, don't wash.
3. Add 100µL of Detection Reagent A working solution to each well. Incubate for 1 hour at 37 °C after covering it with the Plate sealer.
4. Aspirate the solution and wash with 350µL of 1x Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
6. Repeat the aspiration/wash process for five times as conducted in step 4.
7. Add 100µL of Substrate working Solution to each well. Cover with a new Plate sealer. Incubate for 5-10 minutes at 37 °C (Don't exceed 10 minutes). Protect from light.
8. Measure the chemiluminescence signal in a microplate luminometer or as appropriate for the instrument used.

Note:

1. Assay preparation: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20 °C.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between addition of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
5. For Substrate A and B, please protect it from light.
6. Relative light units (RLUs) may differ from different luminometers. The instrument may require settings to be adjusted.
7. Relative light units may vary within the 10 minute reading window.

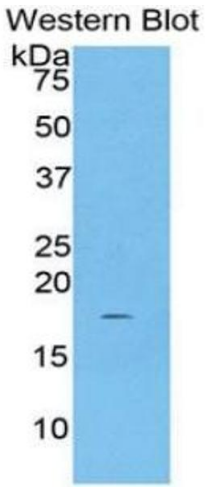
Calculation of Results:	Average the duplicate readings for each standard, control, and samples and subtract the average zero standard relative light unit (RLU). Create a standard curve on log-log graph paper, with target concentration on the y-axis and the RLU value on the x-axis. Draw the best fit

Application Details

	straight line through the standard points and it can be determined by regression analysis. Using some plot software, such as curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. In order to make the calculation easier, we plot the RLU value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is indeed the independent variable while RLU value is the dependent variable. Further, in this part, in order to help the customer perform the assay more visual, we provide the customer with the raw data (not the log of data). However, plotting log of the data to construct the curve will be recommended. The RLU values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). This curve is provided for demonstration only. The customers should establish their own standard curve for each test conducted.
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV < 10% Inter-Assay: CV < 12%
Restrictions:	For Research Use only

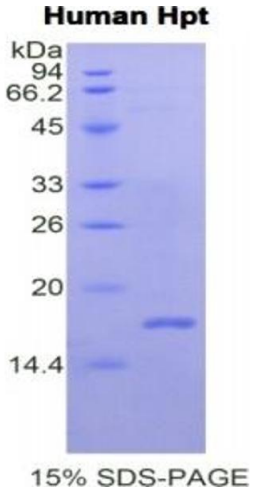
Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C/-20 °C
Expiry Date:	6 months



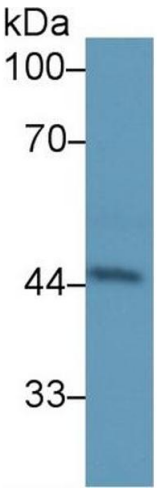
Western Blotting

Image 1. WB of Protein Standard: different control antibodies against Highly purified E. coli-expressed recombinant human Hpt.



SDS-PAGE

Image 2. SDS-PAGE of Protein Standard from the Kit (Highly purified E. coli-expressed recombinant human Hpt).



Western Blotting

Image 3. Rabbit Capture antibody from the kit in WB with Positive Control: Human urine.

Please check the product details page for more images. Overall 4 images are available for ABIN6720463.