HUMAN HEMOPEXIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN HEMOPEXIN CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN HEMOPEXIN ELISA KIT
Catalog No.	SK00515-01
Lot No.:	
Formulation	96 T
Standard range	0.39 – 50 ng/mL
Sensitivity	150 pg/mL
Sample Volume	100 µL
Dilution Factor	*40K-160K (Optimal dilutions should be determined by each laboratory for each application)*
Sample Type	Serum, Plasma
Specificity	Human Hemopexin
Calibration	Human Hemopexin recombinant(HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C up to 1 month, see page 2 for more information
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Human Hemopexin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human HEMOPEXIN from cell culture supernates, serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant human HEMOPEXIN and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural HEMOPEXIN samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human HEMOPEXIN. The capture antibody can bind to the human HEMOPEXIN in the standard and samples. After washing the plate of any unbound substances, an antibody-HRP conjugate against human HEMOPEXIN is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human HEMOPEXIN bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
HEMOPEXIN Microplate – 96 well microplate precoated with a monoclonal anti-human HEMOPEXIN antibody.	**515-01-01**	**1 plate**
HEMOPEXIN Standard – 100 ng/vial of human HEMOPEXIN in a buffered protein base with preservative; lyophilized.	**515-01-02**	**1 vial**
Detection Antibody-HRP Conjugate – 105 µL/vial of 100-fold concentrated solution of antibody conjugated to HRP against HEMOPEXIN.	**515-01-03**	**1 vial**
Positive Control – one vial of human HEMOPEXIN; lyophilized (optional).	**515-01-04**	**1 vial**
Dilution Buffer - 45 mL of buffered protein based solution with preservative.	**DB10**	**2 bottles**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB108A**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control and Dilution Buffer should be stored at -20° C. Detection Antibody-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8° C (DO NOT FREEZE and PROTECT FROM LIGHT). Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.

- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Plasma and serum samples need to be diluted by 40K – 160K. Please refer sample dilution on page 5. **Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

HEMOPEXIN Standard - Reconstitute the HEMOPEXIN standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 100 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **50 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	100 ng/ml
# 1	250µl of stock	250µl	50 ng/ml
# 2	250µl of 1	250µl	25 ng/ml
# 3	250µl of 2	250µl	12.5 ng/ml
# 4	250µl of 3	250µl	6.25 ng/ml
# 5	250µl of 4	250µl	3.125 ng/ml
# 6	250µl of 5	250µl	1.56 ng/ml
# 7	250µl of 6	250µl	0.78 ng/ml
# 8	250µl of 7	250µl	0.39 ng/ml

Positive Control - Reconstitute the positive control with 1 mL of Dilution Buffer to make the 10-fold concentrated positive control stock. Pipette 0.45 mL of Dilution Buffer and transfer 50 µL of 10-fold concentrated stock solution to prepare 1 x working solution of positive control.

Detection Antibody-HRP Conjugate - Pipette 10.395 mL of **Antibody Diluent Solution** into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µl per well of Dilution Buffer to Blank

wells.

4. Add 100 µl per well of standard dilutions from #8 to #S (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 90 minutes on microplate shaker (250 rpm). **Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate Solution to each well. Incubate for 8-13 minutes on microplate shaker at room temperature. **Protect from light**.
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve data is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.045)
0.39	0.036
0.78	0.075
1.56	0.160
3.125	0.326
6.25	0.636
12.5	1.164
25	1.959
50	2.779

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Hemopexin	100
Human Haptoglobin 1	0
Human Haptoglobin 2	0
Human CTRP15	0
Human CRP	0
Human PTX3	0
Human Albumin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody-HRP working solution to each well. Incubate 90 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 8-13 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 minutes.

Use 5 µL of Human serum or plasma samples to prepare 1: 40K ~ 160K dilution.

		Final Dilution
5µL of Human sample	495 µL of 1x Dilution Buffer (DB10)	100
5µL of 100-fold diluted sample solution	495 µL of 1x Dilution Buffer (DB10)	**10000 (10K)**
60 µL of 10000(10K)-fold diluted sample solution	180 µL of 1x Dilution Buffer (DB10)	**40000 (40K)**
30 µL of 10000(10K)-fold diluted sample solution	210 µL of 1x Dilution Buffer (DB10)	**80000 (80K)**
15 µL of 10000(10K)-fold diluted sample solution	225 µL of 1x Dilution Buffer (DB10)	**160000 (160K)**