HUMAN HEMOPEXIN ELISA KIT

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN HEMOPEXIN ELISA
Catalog No.	SK00515-02
Formulation	96 T
Standard Range	1.56-100 ng/ml
Sensitivity	250 pg/mL
Sample Volume	100 µl per well
Sample Type	Serum, plasma
Specificity	Human hemopexin
Sample Dilution	
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	2 °C-8 °C

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 natural human Hemopexin from serum and plasma in a sandwich ELISA format.

This immunoassay contains natural human Hemopexin and antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural human Hemopexin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an 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, a biotinylated detection antibody against human Hemopexin is added to the wells. Following a wash to remove any unbound antibody reagent, a Streptavidin HRP conjugate 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.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
HEMOPEXIN Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human **Hemopexin**.	515-02-01	1 plate
Hemopexin Standard – 100 ng/vial of human **Hemopexin** in a buffered protein base with preservatives; lyophilized.	515-02-02	1 vial
Detection Antibody Concentrated – 1.05mL / vial, 10-fold concentrated of biotinylated Antiobdy against human **Hemopexin** with preservatives;	515-02-03	1 vial
Positive Control- one of human **Hemopexin**, lyophilized	515-02-04	1 vial
Streptavidin HRP Conjugate- 130 µL of 100-fold concentrated Streptavidin HRP Conjugate	SAHRP	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB01	1 vial
HRP Diluent Solution- 12 mL/vial of buffered protein based solution with preservatives	DB06	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution -11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: Reconstituted Detection Antibody and Standard Stock may be stored for up to 1 month at -70°C. Streptavidin HRP conjugate 100 fold concentrated should be stored at 2 - 8° C .
Microplate Wells: Return unused wells to the plastic zip bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.

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

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

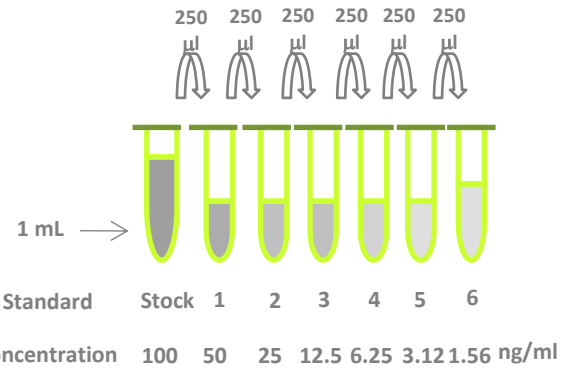
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

Hemopexin Standard – Reconstitute the human Hemopexin standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 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.

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	100 ng/ml
# 1	250 µl of stock	250 µl	50 ng/ml
# 2	250 µl of 1	250 µl	25 ng/ml
# 3	250 µl of 2	250 µl	12.5 ng/ml
# 4	250 µl of 3	250 µl	6.25 ng/ml
# 5	250 µl of 4	250 µl	3.125 ng/ml
# 6	250 µl of 5	250 µl	1.56 ng/ml



Detection Antibody – Reconstitute the Detection Antibody concentrated with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock

solution. Pipette 9.45 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin HRP Conjugate –Pipette 11.88 mL of the appropriate HRP Diluent Solution into the 15 ml centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution.

Positive Control –Reconstitute the Positive Control with 1 mL of Dilution Buffer to prepare working solution.

ASSAY PROCEDURE

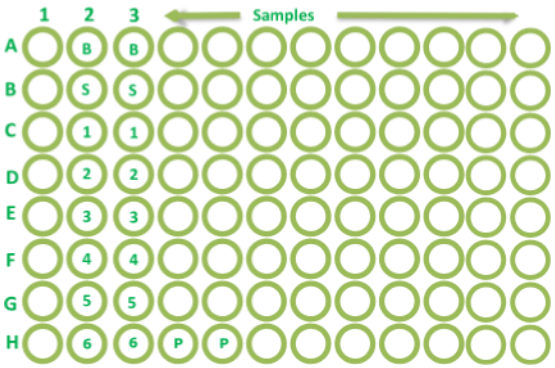
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A2, A3).
4. Add 100 µL of Standard (from B2, B3 to H2, H3,), sample, or control per well (H4, H5). Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin HRP working solution to each well. Incubate for 45 minutes at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 4-7 minutes at room temperature. **Protect from light.**
11. 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.
10. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

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 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.



CALIBRATION

This immunoassay is calibrated against a highly purified human Hemopexin.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Hemopexin was 250 pg/mL.

TYPICAL DATA

These standard curves* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.104)
1.56	0.047
3.125	0.096
6.25	0.187
12.5	0.361
25	0.735
50	1.417
100	2.461

SPECIFICITY

PROTEINS	CROSS-REACTIVITY(%)
Human Hemopexin	100
Human Albumin	0
Human CRP	0
Human MPO	0
Human VDBP	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl of Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl of Streptavidin HRP working solution to each well. Incubate 45 minutes in a paper box (Protect from light) on the plate shaker at RT
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl of Substrate to each well. Incubate 4-7 min on the bench top. Protect from light.
⬇
Add 100 µl of Stop Solution to each well. Read 450nm within 15 min