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# Human Hemopexin ELISA Kit

Catalog # HHPXKT

Strip well format. Reagents for up to 96 tests.

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## INTENDED USE

This human hemopexin (HPX) assay is intended for the quantitative determination of HPX in human plasma, serum, urine, saliva, and milk. **For research use only.**

## BACKGROUND

HPX (aka beta-1B-glycoprotein) is a single chain, 439 amino acid 63 kDa glycoprotein that binds heme with the highest affinity of any known protein [1]. The main function of HPX in the circulation is scavenging heme released by heme containing proteins such as hemoglobin. HPX removes harmful heme and other porphyrin groups to form the second line of defense, after haptoglobin, against hemoglobin-mediated oxidative damage during intravascular hemolysis [2]. Plasma HPX levels can decrease upon injury and inflammation by LRP/CD91-mediated endocytosis of HPX-heme complexes by hepatocytes and macrophages [3].

## ASSAY PRINCIPLE

Human HPX will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled anti-human HPX primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with streptavidin conjugated to HRP. Following an additional washing step, TMB substrate is used for color development at 450nm. Color development is proportional to the concentration of human HPX in the samples. A standard calibration curve is prepared using dilutions of purified HPX and is measured along with the test samples.

## REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human HPX antibody, blocked and dried
- **10X Wash buffer:** 1 bottle of 50ml
- **Human HPX standard:** 1 vial lyophilized standard
- **Anti-human HPX primary antibody:** 1 vial lyophilized antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

## STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

## OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement.
- Manifold dispenser/aspirator or automated microplate washer.
- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes and Pipette tips.
- Deionized or distilled water.
- Polypropylene tubes for dilution of standard.
- Paper towels or laboratory wipes.
- 1N $H_2SO_4$ or 1N HCl.
- Bovine Serum Albumin Fraction V (BSA).
- Tris(hydroxymethyl)aminomethane (Tris).
- Sodium Chloride (NaCl).

## PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

## PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

## SAMPLE COLLECTION

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Centrifuge saliva, milk and urine at 800xg for 10 minutes. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

## ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

### Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/ml standard solution.

Dilution table for preparation of human HPX standard:

| HPX concentration (ng/ml) | Dilutions |
|---|---|
| 100 | 900µl (BB) + 100µl (from vial) |
| 50 | 500µl (BB) + 500µl (100ng/ml) |
| 20 | 600µl (BB) + 400µl (50ng/ml) |
| 10 | 500µl (BB) + 500µl (20ng/ml) |
| 5 | 500µl (BB) + 500µl (10ng/ml) |
| 2 | 600µl (BB) + 400µl (5ng/ml) |
| 1 | 500µl (BB) + 500µl (2ng/ml) |
| 0.5 | 500µl (BB) + 500µl (1ng/ml) |
| 0.2 | 600µl (BB) + 400µl (0.5ng/ml) |
| 0.1 | 500µl (BB) + 500µl (0.2ng/ml) |
| 0 | 500µl (BB) Zero point to determine background |

**NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.**

### Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl HPX standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures HPX antigen in the 0.1-100 ng/ml range. If the unknown is thought to have high HPX levels, dilutions may be made in in blocking buffer. A 1:1,000,000-1:4,000,000 dilution for normal human plasma or serum, 1:100-1:400 dilution for milk, and 1:10-1:40 dilution for urine or saliva is suggested for best results.

### Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1:50,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on a paper towel or a kimwipe.

### Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N $H_2SO_4$ or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

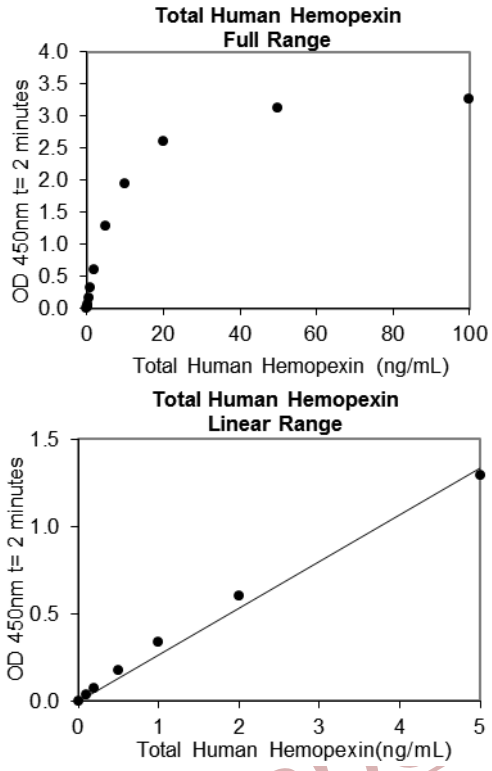
### Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ($A_{450}$).

## Calculation of Results

Plot $A_{450}$ against the amount of HPX in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of HPX in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):




## EXPECTED VALUES

Normal human hemopexin plasma levels range from 0.4-1.5 mg/ml [2]. HPX is reduced in certain disease states such as hemolytic anemia [4], porphyria [5], and sepsis [6].

## PERFORMANCE CHARACTERISTICS

**Sensitivity:** The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range $OD_{450}$: 0.052-0.064) and calculating the corresponding concentration. The MDD was 0.02 ng/mL.

**Intra-assay Precision:** Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| n | 20 | 20 | 20 |
| Mean (ng/ml) | 0.334 | 3.56 | 13.8 |
| Standard Deviation | 0.012 | 0.090 | 0.671 |
| CV (%) | 3.48 | 2.52 | 4.86 |

**Inter-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Recovery:** The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

| Sample | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| n | 4 | 4 | 4 | 4 |
| Mean (ng/ml) | 0.4 | 1.4 | 5.9 | 23.6 |
| Average % Recovery | 101 | 95 | 84 | 94 |
| Range | 100-102% | 91-98% | 83-85% | 76-102% |

**Linearity:** These studies are currently in progress. Please contact us for more information.

**Specificity:** These studies are currently in progress. Please contact us for more information.

**Sample Values:** Samples were evaluated for the presence of the antigen at varying dilutions.

| Sample Type | Dilution | Mean (µg/mL) |
|---|---|---|
| Citrate Plasma | 1:1,000,000 | 805 |
| | 1:2,000,000 | 803 |
| Serum | 1:1,000,000 | 797 |
| | 1:2,000,000 | 760 |
| Urine | 1:20 | 0.043 |
| | 1:40 | 0.043 |
| Saliva | 1:20 | 0.09 |
| | 1:40 | 0.11 |
| Milk | 1:200 | 0.684 |
| | 1:400 | 0.711 |

## DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

## REFERENCES


1. Tolosano E & Altruda F: DNA Cell Biol. 2002, 21:297-306.
2. Delanghe JR & Langlois MR: Clin Chim Acta. 2001, 312:13-23.
3. Hvidberg V, *et al*.: Blood 2005, 106:2572-2579.
4. Muller-Eberhard U, *et al*.: Blood 1968, 32:811-815.
5. Muller-Eberhard U, *et al*.: Proc Soc Exp Biol Med. 1974, 146:694-697.
6. Janz DR, *et al*.: Crit Care. 2013, 17:R272.


**Example of ELISA Plate Layout**
**96 Well Plate: 22 Standard wells, 74 Sample wells**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 0 | 0.1 ng/ml | 0.2 ng/ml | 0.5 ng/ml | 1 ng/ml | 2 ng/ml | 5 ng/ml | 10 ng/ml | 20 ng/ml | 50 ng/ml | 100 ng/ml | |
| **B** | 0 | 0.1 ng/ml | 0.2 ng/ml | 0.5 ng/ml | 1 ng/ml | 2 ng/ml | 5 ng/ml | 10 ng/ml | 20 ng/ml | 50 ng/ml | 100 ng/ml | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |