



AssayMax™

Rat Haptoglobin ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 25 μl of Standard or Sample and 25 μl of Biotinylated Protein per well.
Incubate 2 hours.

Step 2. Wash, then add 50 μl of SP Conjugate per well.
Incubate 30 minutes.

Step 3. Wash, then add 50 μl of Chromogen Substrate per well.
Incubate 7 minutes.

Step 4. Add 50 μl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key



Consult instructions for use.

Rat Haptoglobin ELISA Kit

Catalog No. ERH1003-1

Sample insert for reference use only

Introduction

Haptoglobin (Hpt) is a plasma protein with hemoglobin-binding capacity and plasma glycoproteins that form a stable complex with hemoglobin to aid the recycling of heme iron (1).

Principle of the Assay

The AssayMax Rat Haptoglobin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat haptoglobin in **plasma and serum samples**. This assay employs a quantitative **competitive enzyme immunoassay** technique that measures rat haptoglobin in less than 3 hours. A polyclonal antibody specific for rat haptoglobin has been pre-coated onto a 96-well microplate with removable strips. Haptoglobin in standards and samples is competed with a biotinylated haptoglobin sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Rat Haptoglobin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat haptoglobin.

- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Rat Haptoglobin Standard:** Rat haptoglobin in a buffered protein base (90 µg, lyophilized).
- **Biotinylated Rat Haptoglobin:** 1 vial, lyophilized.
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:250 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Dilute samples 1:250 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents, and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 90 µg of Rat Haptoglobin Standard with 1.5 ml of EIA Diluent to generate a 60 µg/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (60 µg/ml) 1:2 with EIA Diluent to produce 30, 15, 7.5, 3.75, 1.875, and 0.937 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Rat Haptoglobin] (µg/ml)
P1	Standard (60 µg/ml)	60.00
P2	1 part P1 + 1 part EIA Diluent	30.00
P3	1 part P2 + 1 part EIA Diluent	15.00
P4	1 part P3 + 1 part EIA Diluent	7.500
P5	1 part P4 + 1 part EIA Diluent	3.750
P6	1 part P5 + 1 part EIA Diluent	1.875
P7	1 part P6 + 1 part EIA Diluent	0.937
P8	EIA Diluent	0.000

- **Biotinylated Rat Haptoglobin (4x):** Reconstitute Biotinylated Rat Haptoglobin with 4 ml EIA Diluent to produce a 4-fold stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:4 with EIA Diluent. Any remaining solution should be frozen at -20°C and used within 30 days.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of Rat Haptoglobin Standard or sample per well, and immediately add 25 µl of Biotinylated Rat Haptoglobin to each well (on top of the standard or sample) and gently tap plate to mix. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 7 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

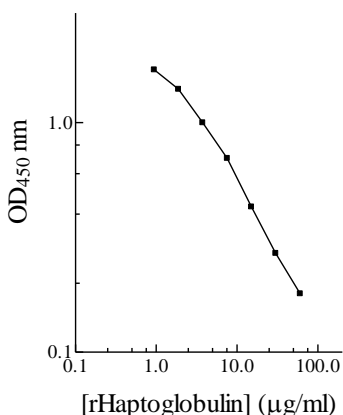
- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

R. Haptoglobin
Standard Curve



Performance Characteristics

- The minimum detectable dose of rat haptoglobin is typically ~ 0.9 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.5% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:125	104%	103%
1:250	100%	97%
1:500	96%	95%

Recovery

Standard Added Value	1.5 – 30 µg/ml
Recovery %	83 – 119%
Average Recovery %	99%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Monkey	<5%
Mouse	<10%
Human	None
Swine	None
Rabbit	None

Reference

- (1) Van Vlierberghe H *et al* (2004) *Clin Chim Acta*. 345(1-2): 35-42

Version 1.3R

Related Products

- EH2003-1 AssayMax Human Haptoglobin ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)
- EH1003-1 AssayMax Human Haptoglobin ELISA Kit (Plasma and Serum samples)
- ERH2003-1 AssayMax Rat Haptoglobin ELISA Kit (Urine and Cell Culture samples)
- EBH2003-1 AssayMax Bovine Haptoglobin ELISA Kit (Urine and Cell Culture samples)
- ECH2003-1 AssayMax Canine Haptoglobin ELISA Kit (Plasma and Cell Culture samples)
- EPH2003-1 AssayMax Swine Haptoglobin ELISA Kit (Plasma, Serum, Urine, and Cell Culture samples)