

# **ab108857**

## **Rat Haptoglobin**

### **ELISA kit**

#### Instructions for Use

For the quantitative measurement of rat Haptoglobin in plasma and serum

This product is for research use only and is not intended for diagnostic use.

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## 1. BACKGROUND

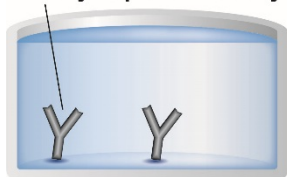
Abcam's Haptoglobin Rat *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Haptoglobin in plasma and serum.

A Haptoglobin specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Haptoglobin is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of haptoglobin captured in plate.

Haptoglobin 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. It is a well-known marker of hemolysis. High Haptoglobin level in plasma was associated with an increased cardiovascular risk in obese men, inflammation, atherosclerosis, and systemic sclerosis.

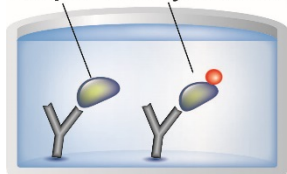
## 2. ASSAY SUMMARY

### Primary Capture Antibody



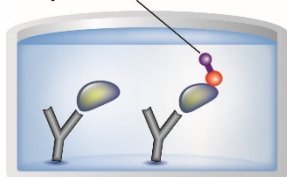
Prepare all reagents, samples and standards as instructed.

### Sample Biotinylated Antigen



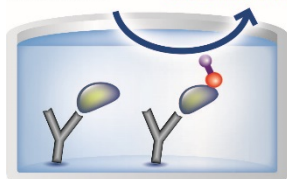
Add standard or sample to each well used and add prepared biotin protein to each well. Incubate at room temperature.

### Streptavidin-HRP



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

### Substrate Colored Product



Wash and add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at 4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Protein, which should be stored at -20°C.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

## 5. MATERIALS SUPPLIED

| Item  | Amount   | Storage Condition (Before Preparation) |
|---|----------|--|
| Haptoglobin Microplate (12 x 8 well strips)           | 96 wells | 4°C                                    |
| Haptoglobin Standard                                  | 1 vial   | 4°C                                    |
| 10X Diluent M Concentrate                             | 30 mL    | 4°C                                    |
| Biotinylated Rat Haptoglobin (Lyophilized)            | 1 vial   | -20°C                                  |
| 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) | 80 µL    | -20°C                                  |
| Chromogen Substrate                                   | 7 mL     | 4°C                                    |
| Stop Solution   | 11 mL    | 4°C                                    |
| 20X Wash Buffer Concentrate                           | 30 mL    | 4°C                                    |
| Sealing Tapes   | 3        | N/A                                    |

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1  $\mu$ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 8 tubes to prepare standard or sample dilutions.

### **7. LIMITATIONS**

- Do not mix or substitute reagents or materials from other kit lots or vendors.

### 8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

## 9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

### 9.1 1X Diluent M

Dilute the 10X Diluent M Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

### 9.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

### 9.3 1X Biotinylated Haptoglobin

Reconstitute the Biotinylated Rat Haptoglobin Protein with 54 ml of Diluent M to generate a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

### 9.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent M.

*Any remaining solution should be frozen at -20°C.*

## 10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
- This procedure prepares sufficient standard dilutions for duplicate wells.

10.1 Reconstitution of the Haptoglobin Standard vial to prepare the 60 µg/mL Haptoglobin **Standard #1**.

10.1.1 First consult the Haptoglobin Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Haptoglobin Standard vial to produce a 60 µg/mL Haptoglobin Standard stock by using the following equation.

$C_S$  = Starting mass of Haptoglobin Standard (see vial label) (µg)

$C_F$  = The 60 µg/mL Haptoglobin **Standard #1** final required concentration

$V_D$  = Required volume of 1X Diluent M for reconstitution (µL)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1,000 = V_D$$

Example:

**NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.**

$C_S$  = 120 µg of Haptoglobin Standard in vial

$C_F$  = 60 µg/mL Haptoglobin **Standard #1** final concentration

$V_D$  = Required volume of 1X Diluent M for reconstitution

$$(120 \mu\text{g} / 60 \mu\text{g/mL}) \times 1,000 = 2,000 \mu\text{L}$$

- 10.1.3 First briefly spin the Haptoglobin Standard vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the Haptoglobin Standard vial by adding the appropriate calculated amount  $V_D$  of 1X Diluent M to the vial to generate the 60  $\mu\text{g/mL}$  **Standard #1**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 60  $\mu\text{g/mL}$  **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- 10.3 Label seven tubes #2 – 8.
- 10.4 Add 120  $\mu\text{L}$  of 1X Diluent M to tube #2 – 8.
- 10.5 To prepare **Standard #2**, add 120  $\mu\text{L}$  of the **Standard #1** into tube #2 and mix gently.
- 10.6 To prepare **Standard #3**, add 120  $\mu\text{L}$  of the **Standard #2** into tube #3 and mix gently.
- 10.7 Using the table below as a guide, prepare subsequent serial dilutions.
- 10.8 1X Diluent M serves as the zero standard, 0  $\mu\text{g/mL}$  (tube #8).

# ASSAY PREPARATION

**Standard Dilution Preparation Table**

| Standard # | Volume to Dilute (μL) | Volume Diluent M (μL) | Total Volume (μL) | Starting Conc. (μg/mL) | Final Conc. (μg/mL) |
|------------|-----------------------|-----------------------|-------------------|------------------------|---------------------|
| 1          | Step 10.1             |                       |                   |                        | 60.0                |
| 2          | 120                   | 120                   | 240               | 60.0                   | 30.00               |
| 3          | 120                   | 120                   | 240               | 30.00                  | 15.00               |
| 4          | 120                   | 120                   | 240               | 15.00                  | 7.500               |
| 5          | 120                   | 120                   | 240               | 7.500                  | 3.750               |
| 6          | 120                   | 120                   | 240               | 3.750                  | 1.875               |
| 7          | 120                   | 120                   | 240               | 1.875                  | 0.938               |
| 8          | -                     | 120                   | 120               | -                      | 0                   |



## 11. SAMPLE PREPARATION

### 11.1 **Plasma**

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes. Dilute samples 1:250 into 1X Diluent M 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.)

### 11.2 **Serum**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes and remove serum. Dilute samples 1:250 into 1X Diluent M and assay. The undiluted serum can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

## 12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

## **13. ASSAY PROCEDURE**

- **Equilibrate all materials and prepared reagents to room temperature (18-25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

- 13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
- 13.2 Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- 13.3 Add 25 µl of Haptoglobin Standard or sample per well, and immediately add 25 µl of Biotinylated Haptoglobin to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- 13.4 Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
- 13.5 Add 50 µL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- 13.6 Wash microplate as described above.
- 13.7 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 10 minutes or till the optimal blue colour density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

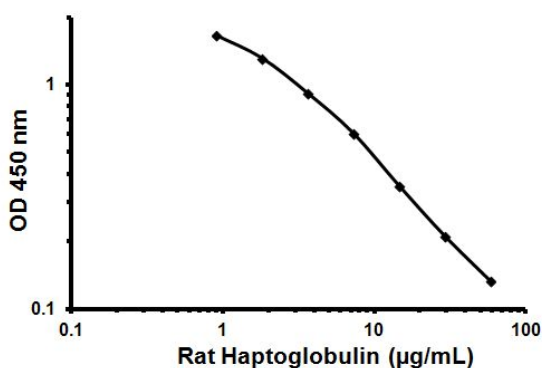
- 13.8 Add 50  $\mu$ L of Stop Solution to each well. The color will change from blue to yellow.
- 13.9 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. 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.

### **14. CALCULATIONS**

Calculate the mean value of the 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.

## 15. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



## 16. TYPICAL SAMPLE VALUES

### SENSITIVITY –

The minimum detectable dose of Haptoglobin is typically 0.56 µg/mL.

### RECOVERY –

Standard Added Value: 1.875 – 15 µg/mL

Recovery %: 86 – 115

Average Recovery %: 98

### LINEARITY OF DILUTION –

| Plasma Dilution | Average % Expected Value |
|-----------------|--------------------------|
| 1:125           | 103                      |
| 1:250           | 99                       |
| 1:500           | 95                       |

| Serum Dilution | Average % Expected Value |
|----------------|--------------------------|
| 1:125          | 105                      |
| 1:250          | 98                       |
| 1:500          | 94                       |

### PRECISION –

|      | Intra-Assay | Inter-Assay |
|------|-------------|-------------|
| % CV | 5.6         | 10.4        |

**17. ASSAY SPECIFICITY**

| <b>Species</b> | <b>% Cross Reactivity</b> |
|----------------|---------------------------|
| Canine         | None                      |
| Monkey         | <5                        |
| Mouse          | <10                       |
| Human          | None                      |
| Swine          | None                      |
| Rabbit         | None                      |
| Bovine         | None                      |
| Equine         | None                      |

## 18. TROUBLESHOOTING

| Problem             | Cause  | Solution  |
|---------------------|--|---|
| Poor standard curve | Improper standard dilution   | Confirm dilutions made correctly  |
|                     | Standard improperly reconstituted (if applicable)  | Briefly spin vial before opening; thoroughly resuspend powder (if applicable)                                 |
|                     | Standard degraded  | Store sample as recommended   |
|                     | Curve doesn't fit scale  | Try plotting using different scale  |
| Low signal          | Incubation time too short  | Try overnight incubation at 4°C   |
|                     | Target present below detection limits of assay   | Decrease dilution factor; concentrate samples   |
|                     | Precipitate can form in wells upon substrate addition when concentration of target is too high | Increase dilution factor of sample  |
|                     | Using incompatible sample type (e.g. serum vs. cell extract)                                   | Detection may be reduced or absent in untested sample types   |
|                     | Sample prepared incorrectly  | Ensure proper sample preparation/dilution   |
| Large CV            | Bubbles in wells   | Ensure no bubbles present prior to reading plate  |
|                     | All wells not washed equally/thoroughly  | Check that all ports of plate washer are unobstructed wash wells as recommended                               |
|                     | Incomplete reagent mixing  | Ensure all reagents/master mixes are mixed thoroughly   |
|                     | Inconsistent pipetting   | Use calibrated pipettes and ensure accurate pipetting   |
|                     | Inconsistent sample preparation or storage   | Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles) |

## RESOURCES

| Problem                             | Cause  | Solution   |
|-------------------------------------|--|--|
| High background/<br>Low sensitivity | Wells are insufficiently washed                              | Wash wells as per protocol recommendations   |
|                                     | Contaminated wash buffer                                     | Make fresh wash buffer   |
|                                     | Waiting too long to read plate after adding STOP solution    | Read plate immediately after adding STOP solution  |
|                                     | Improper storage of ELISA kit                                | Store all reagents as recommended. Please note all reagents may not have identical storage requirements. |
|                                     | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types  |

### 19. NOTES



## Technical Support

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