KAMIYA BIOMEDICAL COMPANY

Rat Hemopexin ELISA

For the quantitative determination of hemopexin in rat biological samples

Cat. No. KT-357

For Research Use Only.

PRODUCT INFORMATION

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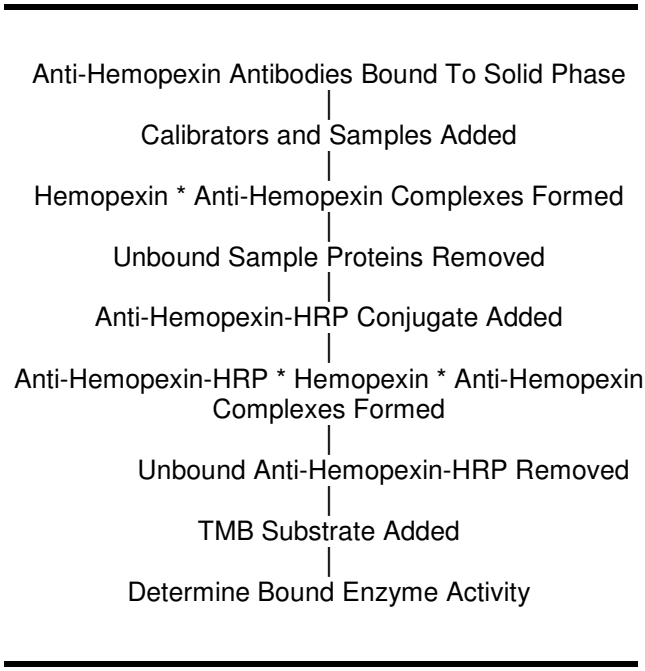
INTENDED USE
The Rat Hemopexin ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of hemopexin in rat biological samples. For research use only.

INTRODUCTION
Hemopexin (HX) is the plasma protein with the highest binding affinity to heme among known proteins. It is mainly expressed in liver and belongs to acute phase reactants, the synthesis of which is induced after inflammation. This ELISA can be used to measure hemopexin in serum, tissue extracts and other biological fluids.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the hemopexin present in the sample reacts with the anti-HX antibodies, which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound sample proteins by washing, anti-HX antibodies conjugated with horseradish peroxidase (HRP), are added. This HRP-conjugated antibody forms a complex with the previously bound hemopexin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is proportional to the concentration of hemopexin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of hemopexin in the test sample. The quantity of hemopexin in the test sample can be interpolated from the calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.



COMPONENTS
1. Diluent Concentrate
   One bottle containing 50 mL of a 5X concentrated diluent running buffer.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 20X concentrated wash solution.

3. Enzyme-Antibody Conjugate Concentrate
   One vial containing 150 µL of a 100X concentrated affinity-purified anti-rat hemopexin antibody conjugated with HRP in a stabilizing buffer.

4. TMB Substrate Solution
   One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

5. Stop Solution
   One vial containing 12 mL of 0.3 M sulfuric acid. WARNING: Avoid contact with skin.

6. Microtiter Plate
   Twelve removable eight-well strips in well holder frame. Wells are coated with affinity-purified anti-rat hemopexin.

7. Rat Hemopexin Calibrator
   One vial containing a lyophilized Rat Hemopexin Calibrator.

8. Positive Control
   One vial containing 50 µL of serum with 0.1% sodium azide. See the Control Certificate for the concentration.

MATERIALS REQUIRED BUT NOT PROVIDED

- Test tubes
- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Preservatives
   - Positive Control contains 0.1% sodium azide.
5. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
6. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
7. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.
   - Do not eat, drink, smoke or apply cosmetics where reagents are used.
   - Avoid all contact with the reagents by using gloves.
   - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent Concentrate
   The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or de-ionized water.

2. Wash Solution Concentrate
   The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate Concentrate
   Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. TMB Substrate Solution
   Ready to use as supplied.

5. Stop Solution
   Ready to use as supplied.

6. Microtiter Plate
   Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

7. Rat Hemopexin Calibrator
   Add 1.0 mL of distilled or de-ionized water to the lyophilized Rat Hemopexin Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 16.4 µg/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Prepare the Rat Hemopexin Calibrators immediately prior to use according to the table below. Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator volume added to 1X Diluent →	Volume of 1X Diluent
1	400	15 µL Rat Hemopexin Calibrator	600 µL
2	200	250 µL Calibrator 1	250 µL
3	100	250 µL Calibrator 2	250 µL
4	50	250 µL Calibrator 3	250 µL
5	25	250 µL Calibrator 4	250 µL
6	12.5	250 µL Calibrator 5	250 µL
7	6.25	250 µL Calibrator 6	250 µL

8. Positive Control
   The concentration and recommended dilution are provided on the Control Certificate. Before use, briefly centrifuge the Positive Control to allow all of the liquid to collect in the bottom of the vial.

STORAGE AND STABILITY

1. Complete Kit
   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long-term storage recommendations below for the Rat Hemopexin Calibrator and Positive Control.**

2. Diluent
   The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

3. Wash Solution
   The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (RT, 16-25°C) or at 4°C.

4. Enzyme-Antibody Conjugate
   Undiluted anti-HX-HRP conjugate should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to eight hours.

5. TMB Substrate Solution
   The TMB Substrate Solution should be stored at 4°C and is stable until the expiration date.

6. Stop Solution
   The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. Microtiter Plate
   Anti-rat hemopexin coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

8. Rat Hemopexin Calibrator
   The lyophilized Rat Hemopexin Calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted and stored frozen. Avoid multiple freeze/thaw cycles. The working calibrator solutions should be prepared immediately prior to use and are stable for up to eight hours at 4°C.

9. Positive Control
   For storage longer than 7 days keep frozen until the expiration date. Storage less than 7 days can be at 4°C. Avoid multiple freeze/thaw cycles.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Serum Samples

Due to the high-sensitive nature of the assay, each serum sample should be diluted before use for a normal assay. A 1:20,000 dilution is appropriate for most serum samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required.

To prepare a 1:20,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X Diluent. This gives you a 1:100 dilution. Next, dilute the 1:100 sample by transferring 5 µL, to 995 µL of 1X Diluent. You now have a 1:20,000 dilution of your sample. Mix thoroughly at each stage.

Procedure

1. Bring all reagents to RT before use.

2. Pipette 100 µL of
   Calibrator 0 (0.0 ng/mL) in duplicate
   Calibrator 1 (15.6 ng/mL) in duplicate
   Calibrator 2 (31.25 ng/mL) in duplicate
   Calibrator 3 (62.5 ng/mL) in duplicate
   Calibrator 4 (125 ng/mL) in duplicate
   Calibrator 5 (250 ng/mL) in duplicate
   Calibrator 6 (500 ng/mL) in duplicate
   Calibrator 7 (1,000 ng/mL) in duplicate

3. Pipette 100 µL of diluted Positive Control (in duplicate) into pre designated wells.

4. Pipette 100 µL of diluted sample (in duplicate) into pre designated wells.

5. Incubate the Microtiter Plate at 22°C (RT) for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.

6. Following incubation, aspirate the contents of the wells.

7. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.

8. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (RT) for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.

9. Wash and blot the wells as described in Steps 6 and 7.

10. Pipette 100 µL of TMB Substrate Solution into each well.

11. Incubate in the dark at RT for precisely ten (10) minutes.

12. After ten minutes, add 100 µL of Stop Solution to each well.

13. Determine the absorbance at 450 nm of the contents of each well. Zero the plate reader to air.

The absorbance of the final reaction mixture can be measured up to two hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from the calibration curve. Correct for sample dilution factor to arrive at hemopexin concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific hemopexin require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

FOR RESEARCH USE ONLY

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