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ab223136
Swine Haptoglobin ELISA Kit

For the quantitative measurement of swine Haptoglobin in serum, plasma, urine and cell culture

samples.

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

Table of Contents

1. Overview

The Swine Haptoglobin ELISA (Enzyme-Linked Immunosorbent Assay) Kit (ab223136) is designed for detection of swine haptoglobin in serum, plasma, urine and cell culture samples.

This assay employs a quantitative sandwich enzyme immunoassay technique that measures swine haptoglobin in approximately 4 hours. A polyclonal antibody specific for swine haptoglobin has been pre-coated onto a 96-well microplate with removable strips. Swine haptoglobin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for swine haptoglobin, which is recognized by a 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.

Haptoglobin (HP, zonulin) is a plasma protein with hemoglobin-binding capacity and a plasma glycoprotein that forms a stable complex with hemoglobin to aid the recycling of heme iron.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells.
Incubate for 2 hours

Wash wells. Add 50 µL Biotinylated Antibody to all wells.
Incubate for 1 hour

Wash wells. Add 50 µL Streptavidin-Peroxidase Conjugate to all wells.
Incubate for 30 minutes

Wash wells. Add 50 µL Chromogen Substrate to all wells.
Incubate for 30 minutes

Add 50 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the Biotinylated Antibody and the Streptavidin-Peroxidase Conjugate, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Swine Haptoglobin coated Microplate (12 x 8 wells)	96 wells	+4°C
Swine Haptoglobin Standard	1 vial	+4°C
Biotinylated Swine Haptoglobin Antibody	1 vial	-20°C
10X Diluent N Concentrate	30 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	7 mL	+4°C
Stop Solution	11 mL	+4°C
Sealing Tapes	3 units	+4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.

8. Technical Hints

- 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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- 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.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.
- When diluting the concentrates, make sure to rinse the bottles thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

9.1 10X Diluent N Concentrate:

Dilute the Diluent N Concentrate 10-fold with reagent grade water. Store for up to 30 days at +4°C.

9.2 Biotinylated Swine Haptoglobin Antibody:

Spin down the antibody briefly before use. The Stock Biotinylated Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Swine Haptoglobin Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Swine Haptoglobin Antibody. The undiluted antibody should be stored at -20°C.

9.3 20X Wash Buffer Concentrate:

Dilute the Wash Buffer Concentrate 20-fold with reagent grade water.

9.4 100X Streptavidin-Peroxidase Conjugate:

Spin down the Streptavidin-Peroxidase Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent N. The undiluted conjugate should be stored at -20°C.

9.5 Anti-Swine Haptoglobin coated Microplate (12 x 8 wells):

Ready to use 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against swine haptoglobin. 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 at +4°C.

9.6 Chromogen Substrate:

Ready to use stabilized peroxidase chromogen substrate tetramethylbenzidine.

9.7 Stop Solution:

Ready to use 0.5 N hydrochloric acid.

9.8 Sealing Tapes:

Ready to use. Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the Swine Haptoglobin Stock to generate a 60 ng/mL **Standard #1**.

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

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the Swine Haptoglobin Standard vial to produce a 60 ng/mL Swine Haptoglobin Standard stock by using the following equation:

CS = Starting mass of Swine Haptoglobin Standard stock (see vial label) (ng)

CF = 60 ng/mL Swine Haptoglobin Standard #1 final required concentration

VD = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_f) * 1{,}000 = V_D$$

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 = 78 ng of Swine Haptoglobin Standard in vial

C_F = 60 ng/mL Swine Haptoglobin **Standard #1** final concentration

V_D = Required volume of 1X Diluent N for reconstitution

(78 ng / 60 ng/mL) * 1,000 = 1,300 µL

10.1.3 First briefly centrifuge the Swine Haptoglobin Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Swine Haptoglobin Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 60 ng/mL Swine Haptoglobin **Standard #1.** Mix gently and thoroughly.

10.2 Allow the reconstituted 60 ng/mL Swine Haptoglobin **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label seven tubes #2 – 8.

10.4 Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (60 ng/mL) 1:2 with equal volume of 1X Diluent N to produce 30, 15, 7.5, 3.75, 1.875, and 0.938 ng/mL solutions. 1X Diluent N serves as the zero standard (0 ng/mL).

10.5 Add 120 µL of 1X Diluent N to tube #2 – 8.

10.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Human Swine Haptoglobin (ng/mL)
1	Step 10.1		60
2	120 µL Standard #1	120	30
3	120 µL Standard #2	120	15
4	120 µL Standard #3	120	7.5
5	120 µL Standard #4	120	3.75
6	120 µL Standard #5	120	1.875
7	120 µL Standard #6	120	0.938
8 (Blank)	N/A	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 3000 x g for 10 minutes and collect plasma. A 160,000-fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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. A 160,000-fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 1500 x g for 10 minutes at 4°C to remove debris and collect supernatants. Dilute samples using 1X Diluent N if necessary.

The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.4 Urine:

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 1:50 (or between 1:5 to 1:500) sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids and cell culture. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (20-25°C).

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 Add 50 µL of Swine Haptoglobin Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 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.

12.5 Add 50 µL of Biotinylated Swine Haptoglobin Antibody to each well and incubate for 1 hour.

12.6 Wash the microplate as described in step12.4.

12.7 Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Turn on the microplate reader and set up the program in advance.

12.8 Wash the microplate as described in step 12.4.

12.9 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 30 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

12.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

12.11 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 high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

12.12 Analyze the data as described below.

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

12.12.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

12.12.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

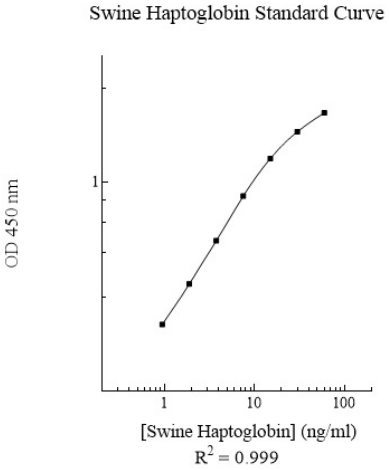

Figure 1. Example of Swine Haptoglobin standard curve in 1X Diluent N. The Haptoglobin standard curve was prepared as described in Section 10.

14. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Swine Haptoglobin as calculated by 2 standard deviations from the mean of a zero standard was established to be 0.40 ng/ml.

PRECISION –

Intra-assay precision was determined by testing replicates of three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-assay Precision	Inter-Assay Precision
Average CV (%)	5.6	10.2

RECOVERY –

Standard Added Value	2-30 ng/ml
Recovery (%)	88 – 111 %
Average Recovery (%)	97%

LINEARITY OF DILUTION

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Dilution Factor	Plasma	Serum
80,000	106%	95%
160,000	100%	114%
320,000	94%	91%

15. Assay Specificity

This kit recognizes Swine Haptoglobin in serum, plasma, urine and cell culture samples.

INTERFERENCES –

10% FBS in culture media will not affect the assay.

16. Species Reactivity

This kit recognizes Swine Haptoglobin.

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Equine	None
Monkey	None
Mouse	None
Rat	None
Rabbit	None
Human	1%

10% FBS in culture media will not affect the assay.

Please contact our Technical Support team for more information.

17. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing

Unexpectedly low or high signal intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted
	Omission of step	Consult the provided procedure for complete list of steps
	Steps performed in incorrect order	Consult the provided procedure for the correct order
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance
	Wash step was skipped	Consult the provided procedure for all wash steps
	Improper wash buffer	Check that the correct wash buffer is being used
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time

Deficient Standard Curve Fit	No-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions

18. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)