Nori® Equine Haptoglobin ELISA Kit DataSheet

Haptoglobin (Hp) is the protein that in humans is encoded by the HP gene. In blood plasma, haptoglobin binds free hemoglobin (Hb) released from erythrocytes with high affinity and thereby inhibits its oxidative activity. The haptoglobin-hemoglobin complex will then be removed by the reticuloendothelial system. In clinical settings, the haptoglobin assay is used to screen for and monitor intravascular hemolytic anemia. In intravascular hemolysis, free hemoglobin will be released into circulation and hence haptoglobin will bind the hemoglobin. This causes a decline in haptoglobin levels. Conversely, in extravascular hemolysis the reticuloendothelial system, especially splenic monocytes, phagocytose the erythrocytes and hemoglobin is not released into circulation; serum haptoglobin levels are therefore normal. Haptoglobin level is used to determine whether hematology needs to be consulted for hemolytic anemia. This gene encodes a preproprotein that is processed to yield both alpha and beta chains, which subsequently combine as tetramer to produce haptoglobin. Haptoglobin functions to bind free plasma hemoglobin, which allows degradative enzymes to gain access to the hemoglobin while at the same time preventing loss of iron through the kidneys and protecting the kidneys from damage by hemoglobin. For this reason it is often referred to as the suicide protein. Haptoglobin is produced mostly by hepatocytes but also by other tissues. In addition, the haptoglobin gene is expressed in murine, human and equine adipose tissue. Haptoglobin consists of two α- and two β-chains, connected by disulfide bridges and exists in two allelic forms in the human population, Hp1 and Hp2, the latter one having arisen due to the partial duplication of Hp1 gene. Hp has been shown to bind hemoglobin with different affinities, with Hp2 being the weakest binder. As haptoglobin is indeed an acute-phase protein, any inflammatory process may increase the levels of plasma haptoglobin.

References

PRINCIPLE OF THE ASSAY

This kit is for quantification of haptoglobin in cattle, cow and bull. This is a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for equine of haptoglobin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and haptoglobin is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for equine of haptoglobin is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of of haptoglobin bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.
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MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Precoated Plate</td>
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<td>20 x PBS</td>
<td>1</td>
<td>Substrate Solution</td>
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<tr>
<td>Detection Antibody</td>
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<td>20 x Assay Buffer</td>
<td>1</td>
<td>Stop Solution</td>
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<td>Conjugate</td>
<td>1</td>
<td>Reagent Diluent</td>
<td>1</td>
<td>DataSheet</td>
<td>1</td>
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<tr>
<td>Standard</td>
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<td>MSDS/CoA</td>
<td>1</td>
<td>96-well plate sheet</td>
<td>1</td>
</tr>
</tbody>
</table>

Bring all reagents to room temperature before use.

Reagent Preparations

Equine Haptoglobin Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C for up to 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of sterile 1 x PBS and vortex 30 sec. If the entire 96-well plate is used, take 200 µL of detection antibody to 10.5 mL of Reagent Diluent to make Working dilution of detection antibody and mix thoroughly prior to the assay. If the partial antibody is used store the rest at -20°C until use.

Equine Haptoglobin Standard (3 vials) – The lyophilized Equine Haptoglobin Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. The unreconstituted standard can be stored at 4°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. Add 500 µL of 1 x Assay Buffer to make the high standard concentration of 200 ng/ml and vortex for 30 sec. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, vortex 30 sec for each of dilution step.

Conjugate (53 µl) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 53 µL Conjugate sufficient for one 96-well plate. If the volume is less than 53 µL, add sterile 1 x PBS to reach 53 µL and vortex 10 sec. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 53 µL of Conjugate to 10.5 mL of Reagent Diluent to make working dilution of Conjugate and mix thoroughly prior to the assay. The rest of undiluted Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Assay Buffer, 20 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
Reagent Diluent, 21 mL.
Substrate Solution, 10.5 mL.
Stop Solution, 5.5 mL.
Nori® Equine Haptoglobin ELISA Kit DataSheet

Assay Procedure
1. Lift the plate cover from the top left and cover the wells that are not used. Vortex briefly the samples prior to the assay. Add 100 μL of sample (such as plasma or serum) or standard per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Assay Buffer, repeating the process one time for a total of two washes. Wash by filling each well with 1 x Assay Buffer (300 μL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Assay Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 μL of the working dilution of Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 μL of the working dilution of Conjugate to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of Substrate Solution to each well. Incubate for 5-20 minutes (depending on signal) at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes
1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A standard curve should be generated for each set of samples assayed. Thorough mixing of standards at each of dilution steps is critical to acquire a normal standard curve.
3. Plasma or serum sample should be diluted with equal volume of 1 x Assay Buffer and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.
4. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
5. The Stop Solution is an acid solution, handle with caution.
6. This kit should not be used beyond the expiration date on the label.
7. A thorough and consistent wash technique is essential for proper assay performance.
8. Use a fresh reagent reservoir and pipette tips for each step.
9. It is recommended that all standards and samples be assayed in duplicate.
10. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.
Nori® Equine Haptoglobin ELISA Kit DataSheet

Calculation of Results
Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the haptoglobin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The Standard Curve
The graph below represents typical data generated when using this equine haptoglobin ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r²) is 0.999-1.000.
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Specificity
The following recombinant equine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP4, BMP7, CRP, CCL2, CCL4, CCL5, HGF, HSP27, IGF-1, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17C, IL-21, IFNγ, PDGF, PLA2G7, serpin E1, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TLR9, TNF-α, TNF RI, TNF RII, VEGF.

Calibration
This kit is calibrated against a highly-purified yeast-expressed recombinant equine haptoglobin.

Detection Range
3-200 ng/ml

Assay Sensitivity
510 pg/ml

Assay Precision
Intra-Assay %CV: 5; Inter-Assay %CV: 8

For Research Use Only.

Related products
20 x Sample Diluent, GR103061
20 x PBS, Cat. GR103004-20
10 x ELISA Wash Buffer, Cat. GR103028
10 x ELISA Reagent Diluent, Cat. GR103055
Universal Blocking Buffer, Cat. GR103005
2 x Recombinant Protein Stabilizer, Cat. GR03014-2
5 x Recombinant Protein Stabilizer, Cat. GR103014-5
ELISA G-Blue Substrate Solution, Cat. GR103021
Recombinant equine haptoglobin, Cat. GR104055-130
Detection antibody
Detection agent
## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>• Inaccurate pipetting</td>
<td>• Check pipettes</td>
</tr>
<tr>
<td></td>
<td>• Improper standard curve</td>
<td>• Check and use the correct dilution buffer</td>
</tr>
<tr>
<td></td>
<td>• OD&lt;sub&gt;450&lt;/sub&gt; too high for the high standard point</td>
<td>• Vortex 30 sec for each of standard dilution steps</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduce substrate incubation time</td>
</tr>
<tr>
<td>Low signal</td>
<td>• Improper preparation of standard, samples, detection antibody, and/or conjugate</td>
<td>• Briefly spin down vials before opening.</td>
</tr>
<tr>
<td></td>
<td>• Too brief incubation times</td>
<td>• Reconstitute the powder thoroughly.</td>
</tr>
<tr>
<td></td>
<td>• Inadequate reagent volume or improper dilution</td>
<td>• Ensure sufficient incubation time including substrate incubation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Check pipettes and ensure correct preparation.</td>
</tr>
<tr>
<td>Large CV</td>
<td>• Inaccurate pipetting and mixing</td>
<td>• Check pipettes and ensure thorough mixing.</td>
</tr>
<tr>
<td></td>
<td>• Improper standard/sample dilutions.</td>
<td>• Use the correct dilution buffers</td>
</tr>
<tr>
<td></td>
<td>• Air bubbles in wells.</td>
<td>• Remove bubbles in wells.</td>
</tr>
<tr>
<td>High background</td>
<td>• Plate is insufficiently washed.</td>
<td>• Review the datasheet for proper wash.</td>
</tr>
<tr>
<td></td>
<td>• Contaminated wash buffer</td>
<td>• If using a plate washer, ensure that all ports are unobstructed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Make fresh wash buffer</td>
</tr>
<tr>
<td>No signal detected</td>
<td>• The procedure was misconducted.</td>
<td>• Ensure the step-by-step protocol was correctly followed and no misstep was conducted.</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>• Improper storage of the ELISA kit</td>
<td>• Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light.</td>
</tr>
<tr>
<td></td>
<td>• Stop solution</td>
<td>• Adding stop solution to each well before reading plate</td>
</tr>
</tbody>
</table>