Nori® Equine IL-6 ELISA Kit-Synovial Fluid DataSheet

IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine and is produced by T cells, macrophages, fibroblasts, osteoblasts, endothelial and other cells (1,2,3). IL-6 induces proliferation and differentiation and acts on B cells, T cells, thymocytes, and others. IL-6 is one of the most important mediators of fever and of the acute phase response. In the muscle and fatty tissue, IL-6 stimulates energy mobilization that leads to increased body temperature. IL-6 can be secreted by macrophages in response to specific microbial molecules, referred to as pathogen associated molecular patterns (PAMPS). IL-6 in concert with TGFβ is important for developing Th17 responses. IL-6 binds to IL-6Rα that through association induces gp130 homodimerization (1). gp130 homodimerization triggers the Jak/STAT cascade and the SHP2/Erk Map kinase cascade (1,4,5). IL-6 also forms a complex with an IL-6Rα splice variant that is non-membrane associated (4). The IL-6/soluble IL-6Rα complex can then activate the gp130 signaling pathway on cells that express gp130 but not IL6Rα (4). IL-6 is relevant to many disease processes such as diabetes (6), atherosclerosis (7), depression (8), Alzheimer's Disease (9), systemic lupus erythematosus (10), prostate cancer (11), breast cancer (12), and rheumatoid arthritis (13).

References

PRINCIPLE OF THE ASSAY

This ELISA kit is for quantification of IL-6 in horse. This is a shorter 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 IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for equine IL-6 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 IL-6 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

Storage
Store at 4°C. The kit should be used in 3 months.

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>
<td>1</td>
<td>20 x PBS</td>
<td>1</td>
<td>Substrate Solution</td>
<td>1</td>
</tr>
<tr>
<td>Detection Antibody</td>
<td>1</td>
<td>20 x Assay Buffer</td>
<td>1</td>
<td>Stop Solution</td>
<td>1</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>1</td>
<td>Reagent Diluent</td>
<td>1</td>
<td>DataSheet</td>
<td>1</td>
</tr>
<tr>
<td>Standard</td>
<td>3</td>
<td>20 x SF Solution</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 IL-6 Detection Antibody (1 vial)** – The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 3 months, if not used immediately. Centrifuge 1 min at 6000 x g to bring down the material prior to open the vial. Each vial contains sufficient Detection Antibody for one 96-well plate. Add 200 µL of sterile 1 x PBS to the antibody vial, vortex 20 sec and allow it to sit for 5 min prior to use. If the entire plate is used, take 200 µL of detection antibody to 10.5 mL of Reagent Diluent if the entire 96-well plate is used 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 IL-6 Standard (3 vial)** – The lyophilized Equine IL-6 Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The un-reconstituted standard can be stored at 4°C ~ -20°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 a Standard vial to make the high standard concentration of 5200 pg/ml and vortex for 20 sec and allow it to sit for 5 min prior to use. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, vortex 20 sec for each of dilution step.

**HRP 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 HRP 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 HRP Conjugate to 10.5 mL of Reagent Diluent to make Working dilution of HRP Conjugate prior to the assay. The rest of undiluted HRP 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.
20 x SF Solution, 10 mL- Dilute to 1 x SF Solution with 1 x PBS prior to use.
Substrate Solution, 10.5 mL.
Stop Solution, 5.5 mL.
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Assay Procedure

1. Lift the plate cover at the top left corner and cover the wells that are not used. Vortex the samples prior to the assay. Add 100 μL of sample (such as SF supernatant, see page 5) or standards per well and use duplicate wells for each 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 two times for a total of three 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 HRP 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 10-20 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 the standards at each step of the dilutions is critical to ensure 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. HRP 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.
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 IL-6 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 IL-6 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 ($r^2$) 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.
ApoAI, BMP1, BMP2, BMP3, BMP4, BMP7, CRP, HGF, HSP27, IL-1α, IL-1 RI, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, sIL-6R, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-21, IL-23, IFNγ, MMP-2, MMP9, IL2R, PDGF, PLA2G7, prolactin, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, VEGF.

Calibration
This kit is calibrated against a highly-purified yeast-expressed recombinant equine IL-6

Detection Range
81-5200 pg/ml

Assay Sensitivity
16 pg/ml

Assay Precision
Intra-Assay %CV: 6; Inter-Assay %CV: 9

Treatment of Synovial Fluid for ELISA assay
1. Add equal volume of 1 x SF Solution to the synovial fluid (SF);
2. Centrifuge for 3 min at 3000 x g to collect supernatant for ELISA assay;
3. If sampled from a rheumatoid arthritis patient or patient who suffered severe inflammatory condition or medication, a serial dilution of the SF supernatant with 1 x SF Solution may be needed for appropriate measurement using Standard Curve generated by the ELISA kit.

For Research Use Only

Related products
ELISA G-Blue Substrate Solution, Cat. 103021
Recombinant equine IL-6, Cat. 104053
Equine IL-6 Detection Antibody
## 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></td>
<td>• Vortex 30 sec for each of standard dilution steps</td>
</tr>
<tr>
<td>Low signal</td>
<td>• Improper preparation of standard, samples, detection antibody, and/or HRP 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.</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 Assay Buffer</td>
<td>• If using a plate washer, ensure that all ports are unobstructed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Make fresh Assay Buffer</td>
</tr>
<tr>
<td>No signal detected</td>
<td>• The procedure was misconduct.</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.</td>
</tr>
<tr>
<td></td>
<td>• Stop solution</td>
<td>• Keep substrate protected from light.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Adding stop solution to each well before reading plate</td>
</tr>
</tbody>
</table>