Nori® Sheep IL-6 ELISA MultiSet Kit 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

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PRINCIPLE OF THE ASSAY

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 Sheep 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 Sheep 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.

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

Storage

Store at 4 °C. The kit can be used in 2 years.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Capture Antibody	1	Detection Antibody	1	Standard	2
CoA	1	DataSheet	1		

Bring all reagents to room temperature before use.

Reagent Preparations

This ELISA MultiSet kit contains sufficient materials for 10 of 96-well plates.

Sheep IL-6 Capture Antibody (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the CoA. Store the vial at -20 °C after reconstitution.

Biotinylated Sheep IL-6 Detection Antibody (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.5 mL of PBS. Dilute in PBS to the working concentration indicated on the CoA. Store the vial at -20 °C after reconstitution.

Sheep IL-6 Standard (2 vials) – The lyophilized Sheep IL-6 Standard has a total of 2 vials. Each vial contains the standard sufficient for generating 5 standard curves. Refer to the lot-specific CoA for the amount supplied. Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Reconstitute each vial with 500 µL of 1 x Assay Buffer. Prepare 500 µL of High Standard per plate assayed at the concentration indicated on the CoA. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, vortex 20 sec for each of dilution step. Store the vial at -20 °C after reconstitution.

Streptavidin-HRP (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. The vial contains 550 µL of streptavidin conjugated to horseradish peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent. DO NOT FREEZE.

Other materials and solutions required but not supplied

1. **PBS**, pH 7.3, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.2 µm filtered.
2. **Assay Buffer**, 0.05% Tween 20 in PBS, pH 7.3.
3. **Reagent Diluent**, 1% bovine serum albumin in PBS, pH 7.3.
4. **Streptavidin-HRP**
5. **Substrate Solution**, mixture of H_2O_2 and tetramethylbenzidine.
6. **Stop Solution**, 1 M H_2SO_4.
7. **96 well microplates with high binding**

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Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody and incubate for 2 hours at room temperature.
2. Aspirate each well and wash with 300 µL of 1 x Assay Buffer per well and remove any remaining Assay Buffer by aspiration or by inverting the plate and blotting it against clean paper towel.
3. Blocks plates by adding 300 µL of 1 x Assay Buffer to each well. Incubate for 1 hour at room temperature.

Assay Procedure

1. Lift the plate cover and cover the wells that are not used using the strip provided. Vortex briefly the samples prior to the assay. Add 100 µL of **samples** (such as plasma or serum) 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 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 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 Standard/Sample diluent 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. The Stop Solution is an acid solution, handle with caution.

5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Assay buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Assay buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. 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 Sheep IL-6 ELISA Kit. The correlation coefficient (r^2) is 0.995-1.000. The standard curve should be calculated using a computer generated 4-PL curve-fit to determine concentrations of unknow specimens.

Specificity

The following recombinant Sheep proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP3, BMP4, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, IL-15, IFNγ, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α.

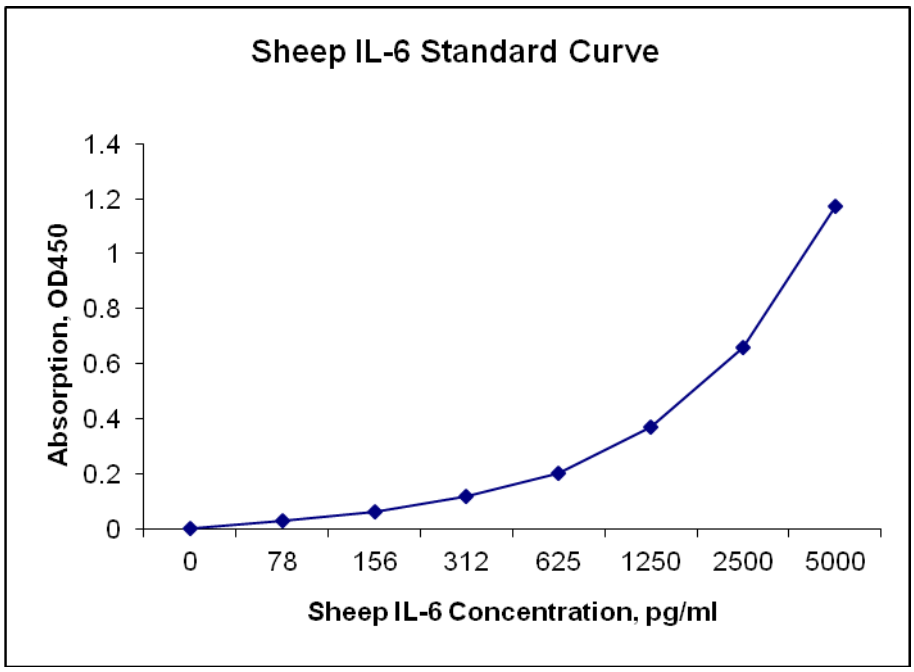
Calibration

This kit is calibrated against a highly-purified yeast-expressed recombinant Sheep IL-6.

Detection Range 78-5000 pg/ml

Assay Sensitivity 15 pg/ml

Assay Precision Intra-Assay %CV: 7; Inter-Assay %CV: 10



Related products

1. GR238016 50 ml Reagent Reservoir, 100/case, 5 packs/case (pack of 20)
2. GR238004 Tissue Culture 96-well Microplate, individually packed, Case of 50
3. GR238019 1.5 ml Microcentrifuge tube with screw cap and free-standing, pack of 500
4. GR238007 125 ml leak-resistant HDPE bottle, colorless, pack of 24
5. GR238002 Microplate 12x8-Well Strip High Binding, Case of 50
6. GR238003 Microplate 12x8-Well Strip Medium Binding, Case of 50
7. GR238032 42592 Costar Stripwell Microplate 1 x 8 Flat Bottom, High Binding, Case of 100
8. GR238001 468667 Thermo Microplate 12x8-Well Strip Nunc Maxixorp F8, Case of 60
9. GR238031 96-well microplate sealer plastic, pack of 100

DECLARATION

THIS REAGENT IS FOR IN VITRO LABORATORY TESTING AND RESEARCH USE ONLY. DO NOT USE IT FOR CLINICAL DIAGNOSTICS. DO NOT USE OR INJECT IT IN HUMANS AND ANIMALS.

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN HUMANS AND ANIMALS

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Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	• Inaccurate pipetting • Insufficient vortexing • OD_{450} too high for the high standard point • Air bubbles in wells. • Standard defect or not fully recovered	• Check pipette calibration and ensure equal dispensing. • Vortex 30 sec for each of standard dilution steps and vortex again (10 sec) before pipetting to the 96-well plate. • Reduce substrate incubation time • Remove air bubbles in wells by pipette tip. • Change a standard vial or spin down the vial before reconstitution
Low signal	• Improper preparation of reagents and storage • Too brief incubation times • Inadequate reagent volume or improper dilution • Standard defect and sample overdiluted	• Briefly spin down vials before opening. Reconstitute the content thoroughly. Proper storage of plate and strip and detection antibody after first usage as shown in the datasheet. Microplate shaker may improve signals. • Ensure sufficient incubation time including substrate incubation. Increase sample incubation to 2 hours. • Change a Standard vial. Undilute sample or less dilution
Overflow in the standards	• Substrate incubation too long • Air bubbles in wells	• Observe the color development every 1-2 mins and reduce substrate incubation time. • Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells. • Remove air bubbles in wells
Large CV	• Inaccurate pipetting and mixing • Improper standard/sample dilutions. • Air bubbles in wells. • Microplate reader out of calibration • It did not turn yellow after adding Stop Solution	• Check pipettes and ensure the pipette is calibrated properly. • Ensure accurate pipetting and thorough mixing. • Use reverse, instead of forward pipetting. • Use the correct dilution buffers • Remove air bubbles in wells by pipette tip. • Calibrate the microplate reader properly • If it did not turn yellow after adding Stop Solution, mix with pipette tip till it turns yellow prior to measurement.
High background	• Reagent reservoir issue • Plate is insufficiently washed and air bubbles in wells. • Contaminated Assay Buffer • Pipet tip contaminated	• Use a new reagent reservoir for Substrate Solution. • Increase wash to 4 times before adding substrate and ensure plate washer functions normally. Remove air bubbles in wells by pipette tip. Use squirt bottle for washing. • Make fresh Assay Buffer and wash thoroughly. • Use new pipette tips for blank wells.
No signal detected	• The procedure was misconducted. • Failures of spin down the contents in Detection Antibody and Standards. • Failure of Substrate or HRP • Samples overdiluted	• Ensure the step-by-step protocol. Spin vials of Detection antibody and Standard to completely recover the content. • Mix 100 µl of Substrate with 0.5 µl HRP and dark blue color should develop in 5 min. • Try a new standard vial and use positive control. • Try not dilute samples
Low sensitivity	• Improper dilutions of standards • Improper storage of the ELISA kit	• Ensure accurate and thorough dilutions of standards at each step. • Store detection antibody at -20°C after reconstitution and others at 4°C. Keep substrate solution protected from light.