GSI Equine IL6 ELISA Kit-Plasma/Serum 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 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 the standard and detection antibody at 4°C ~ -20°C, store 10 x reagent diluents and the antibody pre-coated plate at -20°C and store the rest of the kit at 4 °C. The kit can be used in 3 months.

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

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet	1
Standard	3	96-well plate sheet	1		

Bring all reagents to room temperature before use.

1 x **96-well Plate precoated with equine IL-6 capture antibody-**Store at -20 °C upon received.

Equine IL-6 Detection Antibody– The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for one 96-well plate. Add 200 µL of 1 x Reagent Diluent to the antibody vial, vortex 20 sec and allow it to sit for 5 min prior to use.. Take 200 µL of detection antibody to 9.8 mL Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Equine IL-6 Standard (3 vials) – 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 Reagent Diluent 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 Reagent Diluent, vortex 20 sec for each of dilution step.

Detection Agent (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. The vial contains sufficient detection agent for two 96-well plates. If the volume is less than 50 µL add 1 x Reagent Diluent to a final volume of 50 µL. Make 1:200 dilution in Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 6 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 Wash Buffer, 25 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL – Store at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

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 plasma or serum) or standards per well and use duplicate wells for each sample. Cover the 96-well plate and incubate 2 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash 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 Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the 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 Detection Agent 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 minutes 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. Thorough mixing of the standards at each step of the dilutions is critical to ensure a normal standard curve.
2. If the OD_{450} exceeds the upper limit of the standard curve, dilute the samples with 1 x Wash Buffer and the dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. A standard curve should be generated for each set of samples assayed.
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. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
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.

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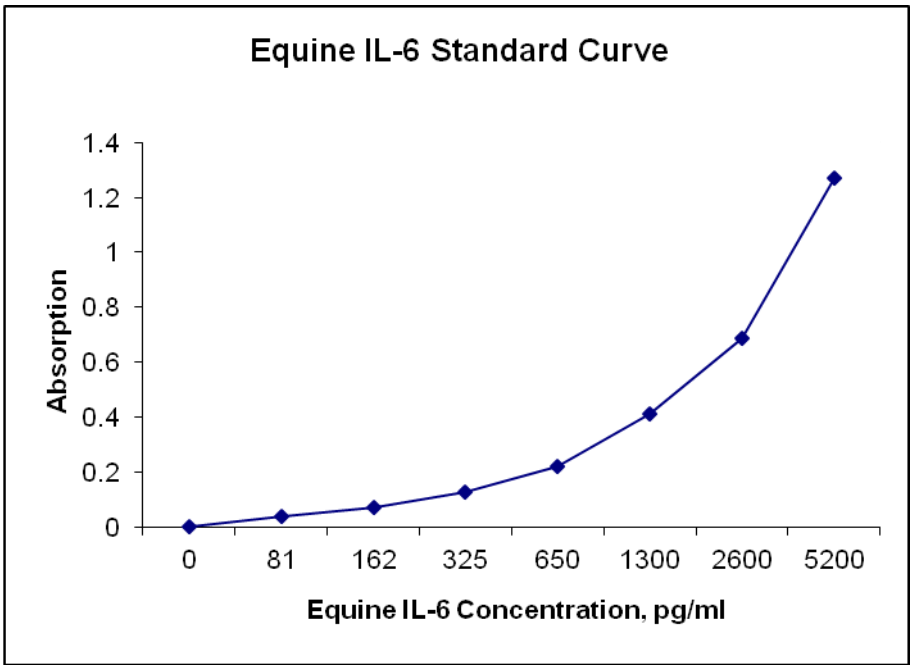
Calculation of Results

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.
BMP1, BMP2, BMP4, HGF, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, sIL-6R, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, MMP-2, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, VEGF.

Calibration

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

Detection Range

1-5200 pg/ml

Assay Sensitivity

0.8 pg/ml

Assay Precision

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

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Related products

20 x Sample Diluent, GRC103058
20 x PBS, Cat. 103004-20
10 x ELISA Wash Buffer, Cat. 103028
10 x ELISA Reagent Diluent, Cat. GRC103055
Universal Blocking Buffer, Cat.103005
2 x Recombinant Protein Stabilizer, Cat. GRC103014-2
5 x Recombinant Protein Stabilizer, Cat. GRC103014-5
ELISA G-Blue Substrate Solution, Cat. 103021
Recombinant equine IL-6, Cat. 104053
Equine IL-6 Detection Antibody