Bovine IL-6 ELISA Kit

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 kit is for quantification of IL-6 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 bovine 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 bovine 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 3 months.

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x TBS	1	Substrate Solution	1
Detection Antibody	1	20 x TBST	1	Stop Solution	1
Conjugate	1	96-well plate sheet	1	DataSheet	1
Standard	3				

Bring all reagents to room temperature before use.

Reagent Preparations

Bovine IL-6 Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C in a manual defrost freezer for up to 3 months, if not used immediately. Spin 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 TBS to the antibody vial, vortex 15 sec and allow it to sit for 5 min prior to use. Take the entire 200 µL of detection antibody to 10.5 mL of 1 x TBST to make **working dilution of Detection Antibody** if the entire 96-well plate is used. If the partial antibody is used store the rest at -20° C until use.

Bovine IL-6 Standard (3 vials) – The lyophilized Bovine IL-6 Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a calibration curve. The unreconstituted standard can be stored at 4°C for up to 3 months if not used immediately. Spin to bring down the material prior to open the tube. Add 500 µL of 1 x TBST to a Standard vial to make the high standard concentration of 4,000 pg /ml. Vortex briefly 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 TBST, vortex 20 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 tube. The vial contains 53 µL conjugate sufficient for a 96-well plate. If the volume is less than 53 µL, add sterile 1 x TBS to reach 53 µL. Make 1:200 dilutions in 1 x TBS. If the entire 96-well plate is used, add 53 µL of Conjugate to 10.5 mL of 1 x TBST to make **working dilution of Conjugate** 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 TBS, pH 7.3, 30 mL- Dilute to 1 x TBS with deionized distilled water and mix well prior to use.
20 x TBST, 20 mL- Dilute to 1 x TBST with 1 x TBS prior to use.
Substrate Solution, 10.5 mL.
Stop Solution, 5.5 mL.

Assay Procedure

1. Lift the plate cover from the top left corner 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 **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 TBST**, repeating the process two times for a total of three washes. Wash by filling each well with 1 x TBST (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 TBST 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 10-20 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. 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 standard at each step of the dilutions is critical to ensure a normal calibration curve.
3. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
4. If sample dilution is needed, 1 x TBST should be used to dilute the samples.
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. TBSt should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining TBSt 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.
11. If dilution is needed, the dilution factors must be used to calculate the concentration. Dilute the sample with 1 x Standard/Sample Diluent.

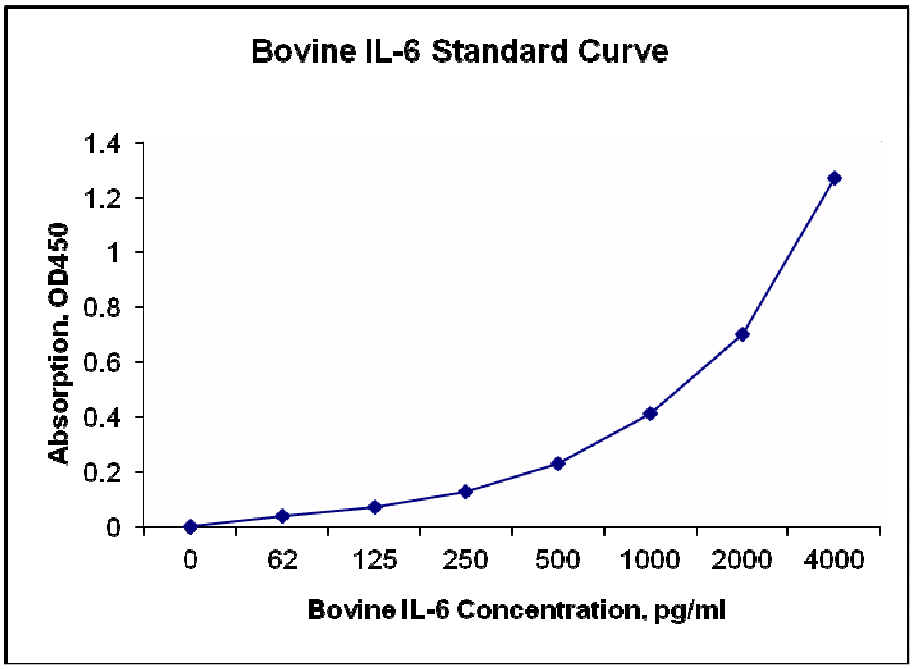
Calculation of Results

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 bovine 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.



Specificity

The following recombinant bovine proteins prepared at 1 ng/ml were tested and exhibited no cross-reactivity or interference.
ApoA1, BMP1, BMP2, BMP3, BMP4, CCL4/MIP-1β, CRP, HSP27, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, IL-15, IL-17C, IL-21, IL-23, IL2R, IL6R, IFNγ, 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 bovine IL-6

Detection Range

62-4,000 pg/ml

Assay Sensitivity

12 pg/ml

Assay Precision

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

For Research Use Only

Related products
20 x TBS, Cat. 103004-20
20 x ELISA TBST, Cat. 103028
10 x ELISA Reagent Diluent, Cat. GR103055
Universal Blocking Buffer, Cat.103005
2 x Recombinant Protein Stabilizer, Cat. GR03014-2
5 x Recombinant Protein Stabilizer, Cat. GR103014-5
ELISA G-Blue Substrate Solution, Cat. 103021
Recombinant bovine IL-6, Cat. 104053
Bovine IL-6 Detection Antibody

Troubleshooting Guide

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