Bovine IL-1β ELISA Kit

Interleukin-1 beta (IL-1β) also known as catabolin, is a member of the interleukin 1 cytokine family. IL-1β precursor is cleaved by caspase 1 (interleukin 1 beta convertase). Cytosolic thiol protease cleaves the product to form mature IL-1β. Interleukin-1 alpha and interleukin-1 beta forms IL-1 (1,2,3).

IL-1β is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. IL-1β gene and eight other interleukin 1 family genes form a cytokine gene cluster on chromosome 2.

References

1. Auron PE, et al (1984). Proc Natl Acad Sci U S A 81 (24): 7907–11.
2. March CJ, et al. (1985). Nature 315 (6021): 641–7.
3. Clark BD, et al (1986). Nucleic Acids Res 14 (20): 7897–1914.

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 Bovine IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Bovine IL-1β 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-1β 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 PBS	1	Substrate Solution, 10.5 mL	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution, 5.5 mL	1
Conjugate	1	10 x Reagent Diluent	1	DataSheet	1
Standard	3	20 x Standard/Sample Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

Bovine IL-1β 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 at 6000 x g for 1 min 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 to the antibody vial, vortex 20 sec and allow it to sit for 5 min. If the entire 96-well plate is used, take 200 µL of detection antibody to 10.5 mL of 1 x Reagent Diluent to make **working dilution of Detection Antibody** and mix thoroughly. If the partial antibody is used store the rest at -20°C until use.

Bovine IL-1β Standard (3 vials) – The lyophilized Bovine IL-1β Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. The un-reconstituted standard can be stored at 4°C to -20°C for up to 3 months if not used immediately. Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Add 500 µL of 1 x Standard/Sample Diluent to a Standard vial to make the high standard concentration of 5000 pg /ml and vortex 20 sec and allow it to sit for 5 min. A seven point standard curve is generated using 2-fold serial dilutions in the Standard/Sample Diluent, vortex 20 sec for each of dilution step.

Conjugate (53 µL) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. The vial contains 53 µL Conjugate sufficient for a 96-well plate. If the volume is less than 53 µL, add sterile 1 x PBS to reach 53 µL. Make 1:200 dilution in 1 x Reagent Diluent. If the entire 96-well plate is used, add 53 µL of Conjugate to 10.5 mL of 1 x Reagent Diluent to make **working dilutions of Conjugate** prior to the assay, and mix thoroughly. The rest of undiluted Conjugate can be stored at 2°C - 8°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 Wash Buffer, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent– Add 3 mL of sterile 1 x PBS to make 10 x Reagent Diluent, vortex 1 min and allow it to sit for 15 min to completely dissolve. Store at -20 °C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
20 x Standard/Sample Diluent, 10 mL- Dilute to 1 x Standard/Sample Diluent with 1 x PBS prior to use.

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 **sample** (such as plasma or serum) or **standards** per well and use duplicate wells for each sample. Cover the 96-well plate and incubate **90 min** 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 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 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. 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.
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.

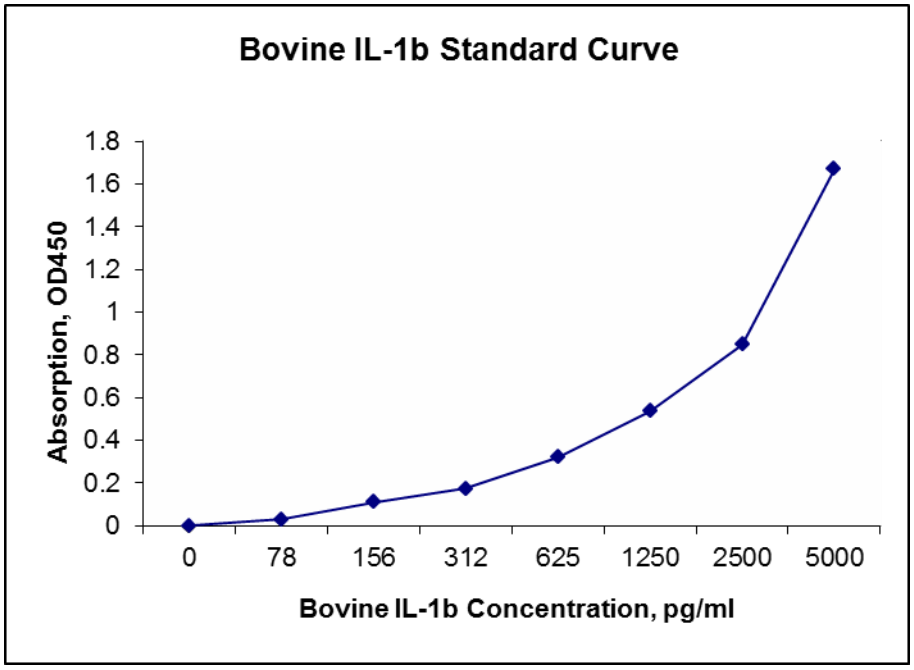
Calculation of Results

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-1β 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-1β 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 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP3, BMP4, IL-2, IL-4, IL-5, IL-6, 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 Bovine IL-1β.

Detection Range

78-5000 pg/ml

Assay Sensitivity

15 pg/ml

Assay Precision

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

For Research Use Only

Related products

10 x ELISA Wash Buffer
10 x Reagent Diluent
20 x PBS
ELISA Substrate
ELISA Stop Solution
ELISA Conjugate
Bovine IL-1β standard
Bovine IL-1β detection antibody.