Bovine IL-1α ELISA Kit

Interleukin-1 alpha (IL-1α) is a protein of the interleukin-1 family that in humans is encoded by the *IL1A* gene.[1] In general, Interleukin 1 is responsible for the production of inflammation, as well as the promotion of fever and sepsis. IL-1α inhibitors are being developed to interrupt those processes and treat diseases. IL-1α is produced mainly by activated macrophages, as well as neutrophils, epithelial cells, and endothelial cells. It possesses metabolic, physiological, haematopoietic activities, and plays one of the central roles in the regulation of the immune responses. IL1A has been shown to interact with HAX1,[2] and NDN.[3] It binds to the interleukin-1 receptor. [4] It is on the pathway that activates tumor necrosis factor-alpha. IL-1α precursor does not contain a signal peptide fragment. After processing by the removal of N-terminal amino acids by specific proteases, primarily by calpain, a calcium-activated cysteine protease.[5] Both the 31kDa precursor and 18kDa mature form of IL-1α are biologically active. It is found in substantial amounts in normal human epidermis and is distributed in a 1:1 ratio between living epidermal cells and stratum corneum.[6] The constitutive production of large amounts of IL-1α precursor by healthy epidermal keratinocytes interfere with the important role of IL-1α in immune responses, assuming skin as a barrier, which prevents the entry of pathogenic microorganisms into the body. The essential role of IL-1α in maintenance of skin barrier function, especially with increasing age,[7] is an additional explanation of IL-1α constitutive production in epidermis. IL-1α has been administered to patients during receiving autologous bone marrow transplantation.[8] The treatment with 50 ng/kg IL-1α from day zero of autologous bone marrow or stem cells transfer resulted in an earlier recovery of thrombocytopenia compared with historical controls. There is currently a head and neck phase III trial being run by Cel-Sci Corp. involving IL-1a and many other interleukins (Multikine) as an immunotherapy.

References

1. March CJ, et al. (1985). *Nature* **315** (6021): 641–7.
2. Yin H, et al. (2001). *Cytokine* **15** (3): 122–37.
3. Hu B, et al. (2003). *Proc. Natl. Acad. Sci. U.S.A.* **100** (17): 10008–13.
4. Bankers-Fulbright JL, Kalli KR, McKean DJ (1996). *Life Sci.* **59** (2): 61–83.
5. Watanabe N, Kobayashi Y (1994). *Cytokine* **6** (6): 597–601.
6. Hauser C, et al. (1986). *J. Immunol.* **136** (9): 3317–23.
7. Barland CO, et al. (2004). *J. Invest. Dermatol.* **122** (2): 330–6.
8. Smith JW, et al. (1993). *N. Engl. J. Med.* **328** (11): 756–61.

PRINCIPLE OF THE ASSAY

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

Bring all reagents to room temperature before use.

Reagent Preparations

Bovine IL-1α 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 PBS 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 mL of 1 x PBS 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-1α Standard (3 vials) – The lyophilized Bovine IL-1α 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 Standard/Sample Diluent to a Standard vial to make the high standard concentration of 200 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 Standard/Sample Diluent, vortex 20 sec for each of dilution step.

Conjugate (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. The vial contains 50 µL conjugate sufficient for a 96-well plate. If the volume is less than 50 µL, add sterile 1 x PBS to reach 50 µL. Make 1:200 dilutions in 1 x PBS. If the entire 96-well plate is used, add 50 µL of Conjugate to 10 mL of 1 x PBS 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 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 with 1 x PBS prior to use.
Substrate Solution, 10 mL.
Stop Solution, 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 **2 hours** 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 **2 hours** 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 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 Standard/Sample Diluent 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. 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.
11. If dilution is needed, the dilution factors must be used to calculate the concentration. Dilute the sample with 1 x Standard/Sample Diluent.

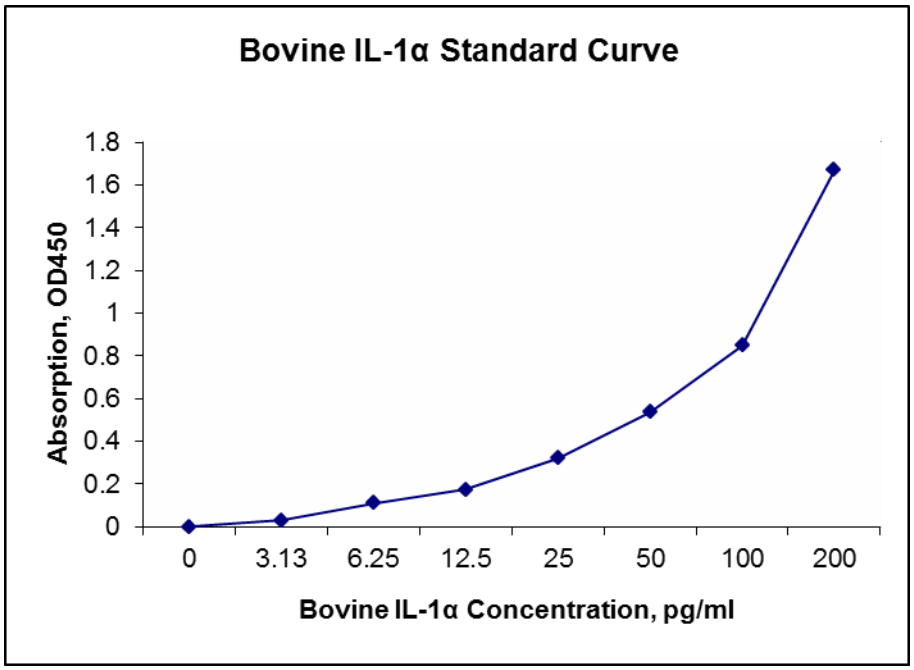
Calculation of Results

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-1α 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-1α 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, IL-1 ALPHAR, 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-1α

Detection Range

3-200 pg/ml

Assay Sensitivity

1 pg/ml

Assay Precision

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

For Research Use Only

Related products
20 x PBS
10 x ELISA Wash Buffer
10 x ELISA Reagent Diluent
Universal Blocking Buffer
2 x Recombinant Protein Stabilizer
5 x Recombinant Protein Stabilizer
ELISA G-Blue Substrate Solution
Recombinant bovine IL-1α
Bovine IL-1α Detection Antibody