Bovine IL-4 ELISA Kit

Interleukin-4, abbreviated IL-4, is a cytokine that is expressed in a variety of tissues including lymphocytes and leukocytes, is a key regulator in humoral and adaptive immunity and has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of CD4+ T-cells into Th2 cells. IL-4 induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4. The cell that initially produces IL-4, thus inducing Th0 differentiation, has not been identified, but recent studies suggest that basophils may be the effector cell.[1] It is closely related and has functions similar to Interleukin 13.

Like IL-13, Interleukin 4 (IL-4) is critical for responses to parasitic helminthes. [2] IL-4 up-regulates MHC class II production and decreases the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell IL-12. Tissue macrophages play an important role in chronic inflammation and wound repair. The presence of IL-4 in extravascular tissues promotes alternative activation of macrophages into M2 cells and inhibits classical activation of macrophages into M1 cells. An increase in repair macrophages (M2) is coupled with secretion of IL-10 and TGF-β that result in a diminution of pathological inflammation. This cytokine was co-discovered by Maureen Howard and William Paul[3] and by Dr. Ellen Vitetta and her research group in 1982.

References

1. Sokol, C.L., Barton, G.M., Farr, A.G. & Medzhitov, R. (2008). "A mechanism for the initiation of allergen-induced T helper type 2 responses". *Nat Immunol* **9** (3): 310–318.
2. Liang, H-E, Reinhardt, R.L., Bando, J.K., Sullivan, B.M., Ho, I-C, & Locksley, R.M. (2012) Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nature Immunology, 13: 58–66.
3. Howard M, Paul WE (1982). "Interleukins for B lymphocytes". *Lymphokine Res.* **1** (1): 1–4.

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 Bovine IL-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Bovine IL-4 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-4 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 the standard, detection antibody, 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. The pre-coated plate can also be stored at 4 °C if used in a month.

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/Manual	1
Standard	3			96-well plate sheet	1

Bring all reagents to room temperature before use.
1 x **96-well Plate precoated with Bovine IL-4 capture antibody-**Store at -20°C upon received.

Bovine IL-4 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 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g 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 1 x Reagent Diluent to the antibody vial and vortex briefly and sit for 5 min. Take 200 µL of detection antibody to 9.8 mL 1 x Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Bovine IL-4 Standard (3 vials) – The lyophilized bovine IL-4 Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The non-reconstituted standard can be stored at 4°C to -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 1 x Reagent Diluent to one Standard vial to make the high standard concentration of 2500 pg /ml. Vortex 20 sec and allow it to sit for a minimum of 15 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. Store the rest of the standard at -20°C.

Detection Agent (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. If the volume is less than 50 µL, add 1 x Reagent Diluent to a final volume of 50 µL and vortex briefly. Make 1:200 dilutions 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, 20 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 and mix well.
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

Assay Procedure

1. Lift the plate cover 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 standard per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 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 5-10 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 standard at each dilution step is critical to ensure the normal standard curve.
2. If IL-4 exceeds the upper limit of the detection, the sample needs to be diluted with 1 x Wash Buffer. 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.

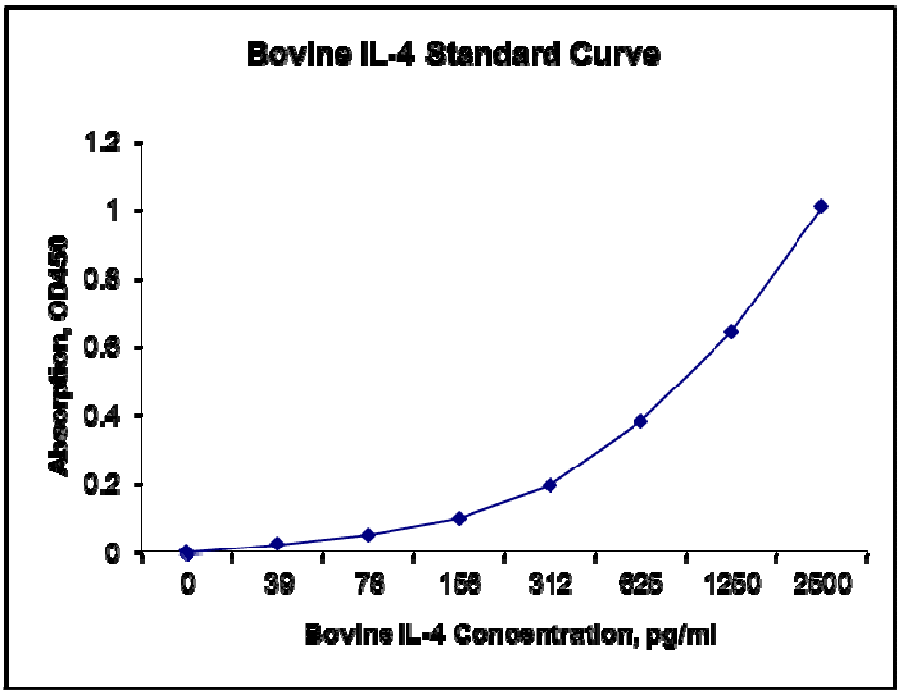
Calculation of Results

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-4 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-4 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.
BMP1, BMP2, BMP3, BMP4, HGF, HSP27, IL-1β, IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, IL-17C, IL-23, MMP-2, MMP9, Prolactin, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, VEGF.

Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant bovine IL-4.

Detection Range

39-2500 pg/ml

Assay Sensitivity

1.6 pg/ml

Assay Precision

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

For Research Use Only

Related products

ELISA G-Blue Substrate Solution
Bovine IL-4 Standard
Bovine IL-4 detection antibody
EIA Buffer