# Bovine IL-17C ELISA Kit

Interleukin 17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation. IL-17 is produced by T-helper cells and is induced by IL–23 which results in destructive tissue damage in delayed-type reactions (1). Interleukin 17 as a family functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. Interleukin 17 acts synergistically with tumor necrosis factor and interleukin-1 (2). To elicit its functions, IL-17 binds to a type I cell surface receptor called IL-17R of which there are at least three variants IL17RA, IL17RB, and IL17RC (3).

The IL-17 family is comprised of at least six proinflammatory cytokines that share a conserved cysteine-knot structure but diverge at the N-terminus. In addition to IL-17A, members of the IL-17 family include IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F.

IL-17 family members are glycoproteins secreted as dimers that induce local cytokine production and recruit granulocytes to sites of inflammation. IL-17 is induced by IL-15 and IL-23, mainly in activated CD4+ T cells distinct from Th1 or Th2 cells. IL-17F is the most homologous to IL-17, but is induced only by IL-23 in activated monocytes. IL-17B binds the IL-17B receptor, but not the IL-17 receptor; it is most homologous with IL-17D, which is expressed by resting CD4+ T cells and CD19+ B cells. IL-17E is mainly produced by Th2 cells and recruits eosinophils to lung tissue. IL-17C has a very restricted expression pattern but has been detected in adult prostate and fetal kidney libraries.

**References**

1. Janis, K, et al. (2007). Kuby immunology. San Francisco: W.H. Freeman. pp. 396.
2. Miossec P, et al. (2009). N. Engl. J. Med. 361 (9): 888.
3. Starnes T, et al. (2002). J. Immunol. 169 (2): 642.

## 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 and the sensitivity of the assays. An antibody specific for bovine IL-17C has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-17C present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for bovine IL-17C 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-17C 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 and detection antibody at 4°C or -20°C, 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.

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 | 20 x Standard/Sample Diluent | 1 | 96-well plate sheet | 1 |

Bring all reagents to room temperature before use.
1 x **96-well Plate precoated with Bovine IL-17C antibody-**Store at 4°C or -20°C upon receipt.

**Bovine IL-17C Detection Antibody**– The lyophilized Detection Antibody should be stored at 4°C or -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 100 µL of sterile 1 x PBS to the antibody vial, vortex 20 sec and allow it to sit for 5 min. Take 200 µL of detection antibody to 9.9 mL of 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-17C Standard** (3 vials) – The lyophilized bovine IL-17C Standard has 3 vials. Each vial contains the standard sufficient for generating a standard curve. The non-reconstituted standard can be stored at 4°C or -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 vial. Add 500 µL of 1 x Standard/sample Diluent to a standard vial to make the high standard concentration of 8000 pg /ml. Vortex 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 the Standard Diluent, vortex 20 sec for each of dilution steps.

**Detection Agent** (1 vial) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Agent for a 96-well plate. Add 100 µL of sterile 1 x PBS to the vial and vortex 1 min and allow it to sit for 5 min. Make 1:100 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 100 µL of Detection Agent to 9.9 mL of Reagent Diluent prior to the assay. The rest of undiluted Detection Agent 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. Stored at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
**20 x Standard/sample Diluent**, 10 mL
**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 and the standards prior to the assay. Add 100 µL of sample (such as plasma or serum) or standard to each well, use duplicate wells for each of the standards and samples, 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 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. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standards at each of dilution steps is critical to ensure a normal standard curve.
2. If IL-17C 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. 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. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

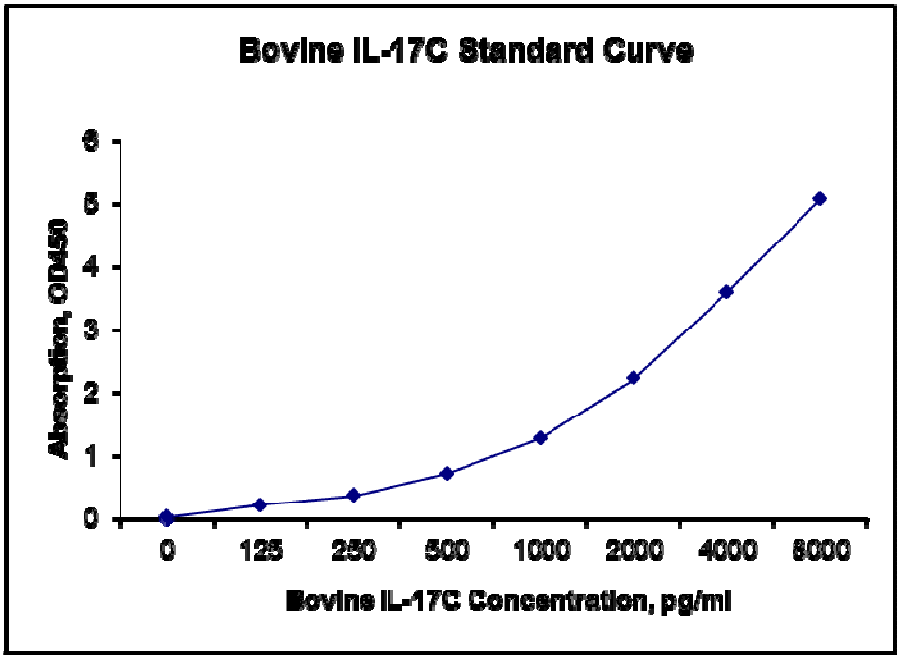
## Calculation of Results

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-17C 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-17C ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark$^{TM}$ 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, BMP4, HGF, IL-1β, IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, MMP-2, sIL-2R, sIL-6R, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, VEGF.

## Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant bovine IL-17C.

## Detection Range

125-8000 pg/ml

## Assay Sensitivity

23 pg/ml

## Assay Precision

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

# For Research Use Only

## Related products

20 x Sample Diluent
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
Bovine IL-17C Standard
Bovine IL-17C detection antibody