# Bovine IL-12 ELISA Kit

Interleukin 12 (IL-12) is the founding member of the IL-12 family of heterodimeric cytokines, which have important immunological functions. IL-12 is composed of two disulfidelinked subunits of 35 kDa and 40 kDa, respectively. The 35 kDa subunit (p35) is an αhelical protein homologous to IL6 and GCSF.The 40 kDa subunit (p40) contains one fibronectin type III and one Ig C2like domain, and has a high degree of structural homology to type I cytokine receptors. Whereas p35 subunit is unique to IL-12, the p40 subunit is also utilized in IL-23. Mature rat p40 contains 313 aa and can exist in multiple forms, including monomer, homodimer, heterodimer linked to p19 (forming IL-23), and heterodimer linked to p35 (forming IL-12). The expression of p40 is upregulated by substances such as LPS and CpG that activate antigenpresenting cells.

Cells known to produce IL-12 include macrophages, dendritic cells, monocytes, Langerhans cells, neutrophils, and keratinocytes. The activities of IL-12 are mediated by the receptor complex composed of two type I transmembrane proteins: a 100 kDa ligandbinding subunit (IL-12 Rβ1) and a 130 kDa signal transducing subunit (IL-12 Rβ2). IL-12 mediates enhancement of the cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes, and potentiates the expansion and late activation of Th1 CD4+ T cells. IL-12 also has anti-angiogenic activity (1-4). IL-12 is linked with autoimmunity, and normally stops allergies to food developing (5).

**References**

1. Park, A.Y. and P. Scott (2001) Scand. J. Immunol. 53:529.
2. Trinchieri, G. et al. (2003) Immunity 19:641.
3. Brombacher, F. et al. (2003) Trends Immunol. 24:207.
4. Lankford, C.S. and D.M. Frucht (2003) J. Leukoc. Biol. 73:49
5. Temblay JN, et al. J Allergy Clin Immunol. 2007 Sep;120(3):

## 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-12 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-12 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for bovine IL-12 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-12 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 |
| Detection Antibody | 1 | 10x Reagent Diluent | 1 | DataSheet | 1 |
| Standard | 3 | 96-well plate sheet | 1 | | |

Bring all reagents to room temperature before use.

**Reagent Preparations**

1 x **96-well Plate precoated with bovine IL-12 capture antibody**-Store at -20°C upon receipt.

**Bovine IL-12 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 at 5000 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 1 x Reagent Diluent to the antibody vial and allow it to sit for 5 min and centrifuge for 1 min at 5000 x g. Take 200 µL of detection antibody to 9.8 mL 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-12 Standard** (3 vials) – The lyophilized Bovine IL-12 Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The unreconstituted standard can be stored at 4°C ~ -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 Reagent Diluent to a Standard vial to make the high standard concentration of 5000 pg /ml. Vortex 20 sec and allow it to sit for a minimum of 5 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.

**Detection Agent** (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. The vial contains sufficient detection agent for a 96-well plate. If the volume is less than 50 µL, add 1 x Reagent Diluent to make a final volume of 50 µL. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add all 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.
**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 1 hour at room temperature.
2. Aspirate each well and wash with **1x 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 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. 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.
2. If IL-12 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.

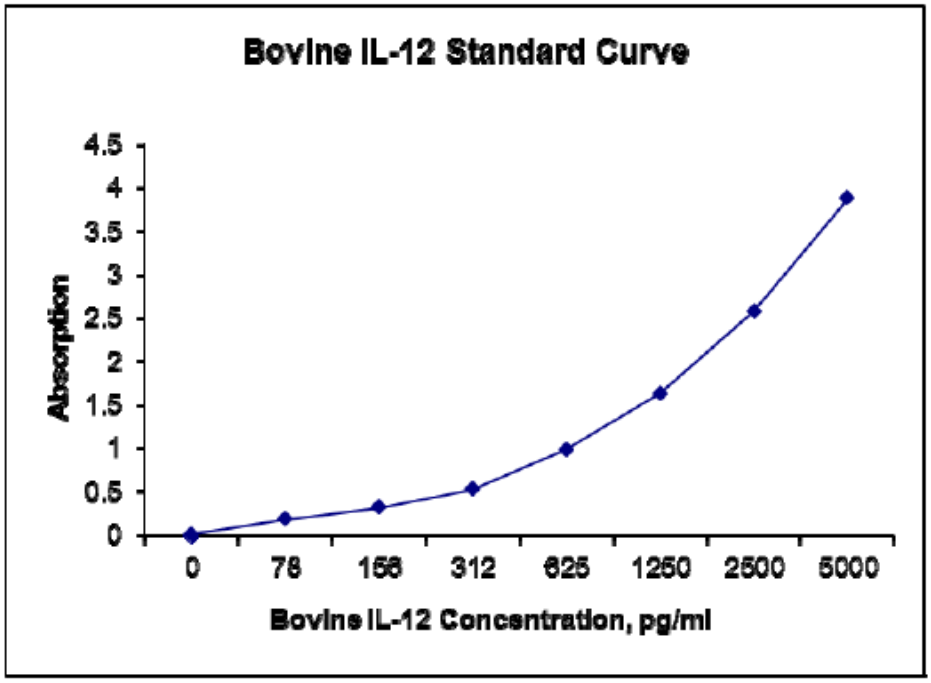
## Calculation of Results

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-12 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-12 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-13, 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-12

## Detection Range

78-5,000 pg/ml

## Assay Sensitivity

2 pg/ml

## Assay Precision

Intra-Assay %CV: 5; Inter-Assay %CV: 9

# For Research Use Only

**Related products**

10 x ELISA Wash Buffer
10 x Reagent Diluent
20 x PBS
ELISA Substrate
ELISA Stop Solution
ELISA Detection Agent
Bovine IL-12 standard
Bovine IL-12 detection antibody

**Troubleshooting Guide**

| Problem | Possible causes | Solution |
|---|---|---|
| Poor standard curve | • Inaccurate pipetting<br>• Improper standard curve | • Check pipettes<br>• Check and use the correct dilution buffer<br>• Vortex 30 sec for each of standard dilution steps |
| Low signal | • Improper preparation of standard, samples, detection antibody, and/or conjugate<br>• Too brief incubation times<br>• Inadequate reagent volume or improper dilution | • Briefly spin down vials before opening. Reconstitute the powder thoroughly.<br>• Ensure sufficient incubation time.<br>• Check pipettes and ensure correct preparation. |
| Large CV | • Inaccurate pipetting and mixing<br>• Improper standard/sample dilutions.<br>• Air bubbles in wells. | • Check pipettes and ensure thorough mixing.<br>• Use the correct dilution buffers<br>• Remove bubbles in wells. |
| High background | • Plate is insufficiently washed.<br>• Contaminated wash buffer | • Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed.<br>• 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<br>• Stop solution | • Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light.<br>• Adding stop solution to each well before reading plate |