Nori® Bovine IL-8 ELISA MultiSet Kit DataSheet

IL-8 was renamed CXCL8 by the Chemokine Nomenclature Subcommittee of the International Union of Immunological Societies (1), although its approved HUGO gene symbol remains IL8. CXCL8 was also referred to as neutrophil chemotactic factor (NCF), neutrophil activating protein (NAP), monocyte-derived neutrophil chemotactic factor (MDNCF), T lymphocyte chemotactic factor (TCF), granulocyte chemotactic protein (GCP) and leukocyte adhesion inhibitor (LAI). Many cell types, including monocyte/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, chondrocytes, and various tumor cell lines, can produce CXCL8 in response to a wide variety of proinflammatory stimuli such as exposure to IL1, TNF, LPS, and viruses. CXCL8 is a potent chemoattractant for neutrophils. In addition, CXCL8 also has a wide range of other proinflammatory effects. CXCL 8 is also a potent angiogenic factor. CXCL8 causes degranulation of neutrophil specific granules and azurophilic granules. CXCL8 induces expression of the cell adhesion molecules CD11/CD18 and enhances the adherence of neutrophils to endothelial cells and subendothelial matrix proteins. Besides neutrophils, CXCL8 is also chemotactic for basophils, T cells and eosinophils. CXCL8 has been reported to be a comitogen for keratinocytes and was also shown to be an autocrine growth factor for melanoma cells. It is a key parameter in localized inflammation (2). It has been cited as a proinflammatory mediator in gingivitis and psoriasis (3).

References

1. Bacon K, et al (October 2002). J. Interferon Cytokine Res. 22 (10): 1067–8.
2. Vlahopoulos S, et al. (September 1999). *Blood* **94** (6): 1878–89.
3. Utgaard JO, et al (November 1998). *J. Exp. Med.* **188** (9): 1751–6.

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-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Bovine IL-8 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-8 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 2 years.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Capture Antibody	1	Detection Antibody	1	Standard	3
Streptavidin-HRP	1	MSDS/CoA	1	DataSheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

This ELISA MultiSet kit contains sufficient materials for 10 of 96-well plates.

Bovine IL-8 Capture Antibody (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.55 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the CoA. Store the vial at -20 °C after reconstitution.

Biotinylated Bovine IL-8 Detection Antibody (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.55 mL of PBS. Dilute in PBS to the working concentration indicated on the CoA. Store the vial at -20 °C after reconstitution.

Bovine IL-8 Standard (3 vials) – The lyophilized Bovine IL-8 Standard has a total of 3 vials. Each vial contains the standard sufficient for generating 15 standard curves. Refer to the lot-specific CoA for the amount supplied. Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Reconstitute each vial with 200 µL of Assay Buffer. Prepare 500 µL of High Standard per plate assayed at the concentration indicated on the CoA with Assay Buffer. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, vortex 20 sec for each of dilution step. Store the vial at -20 °C after reconstitution.

Streptavidin-HRP (1 vial) – Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. The vial contains 550 µL of streptavidin conjugated to horseradish peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent. DO NOT FREEZE.

Other materials and solutions required but not supplied

1. **PBS**, pH 7.3, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.2 µm filtered.
2. **Assay Buffer**, 0.05% Tween 20 in PBS, pH 7.3.
3. **Reagent Diluent**, 1% bovine serum albumin in PBS, pH 7.3.
4. **Substrate Solution**, mixture of H_2O_2 and tetramethylbenzidine.
5. **Stop Solution**, 1 M H_2SO_4.
6. **96-well microplates with high binding.**

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Plate preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody and incubate for 2 hours at room temperature.
2. Aspirate each well and wash with 300 µL of Assay Buffer per well and remove any remaining Assay Buffer by aspiration or by inverting the plate and blotting it against clean paper towel.
3. Blocks plates by adding 150 µL of Reagent Diluent to each well. Incubate for 1 hour at room temperature.
4. Wash the plate as shown in Step 2.

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 **1 hour** at room temperature.
2. Aspirate each well and wash with **Assay Buffer**, repeating the process two times for a total of three washes. Wash by filling each well with Assay 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 Assay 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 5-20 minutes (**depending on signal**) 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. Assay buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Assay 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-8 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-8 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, IFNγ, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α.

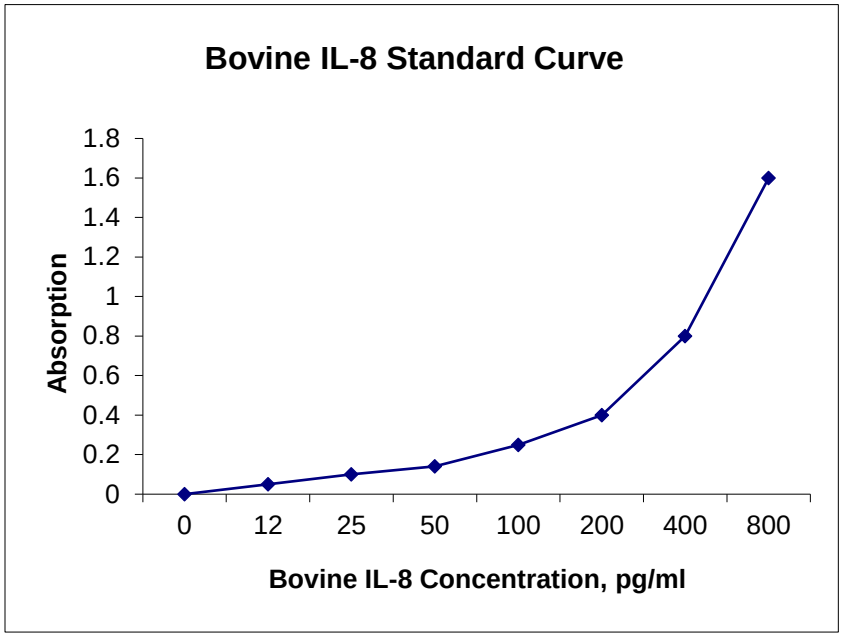
Calibration

This kit is calibrated against a highly-purified yeast-expressed recombinant Bovine IL-8.

Detection Range 12-800 pg/ml

Assay Sensitivity 2 pg/ml

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



For Research Use Only

Related products
20 x ELISA Assay Buffer, GR103014
10 x Reagent Diluent, GR103028
20 x PBS, GR103004-20
ELISA Substrate, GR103021
ELISA Stop Solution, GR103055
ELISA Conjugate, GR103044
Bovine IL-8 standard
Bovine IL-8 detection antibody

Troubleshooting Guide

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