Nori® Canine IFNβ ELISA MultiSet Kit DataSheet

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells, and are very important for fighting viral infections. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors. IFNs belong to the large class of glycoproteins known as cytokines and are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: activation of immune cells, such as natural killer cells and macrophages; increasing recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes; and increasing the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection. About ten distinct IFNs have been identified in mammals and are typically divided among three IFN classes: Type I, Type II and Type III IFN and IFN-β belongs to type I IFNs bind to a specific cell surface receptor complex known as the IFN-α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains.[1] In addition to the JAK-STAT pathway, IFNs can activate several other signaling cascades. Both type I and type II IFNs activate a member of the CRK family of adaptor proteins called CRKL, a nuclear adaptor for STAT5 that also regulates signaling through the C3G/Rap1 pathway.[2] Type I IFNs further activate *p38 mitogen-activated protein kinase* (MAP kinase) to induce gene transcription.[2] The immune effects of interferons have been exploited to treat several diseases. Interferon beta-1a and interferon beta-1b are used to treat and control multiple sclerosis, an autoimmune disorder. This treatment is effective for slowing disease progression and activity in relapsing-remitting multiple sclerosis and reducing attacks in secondary progressive multiple sclerosis.[3]

References

1. de Weerd NA, et al. (2007). *J Biol Chem* **282** (28): 20053–20057.
2. Platanias, L. C. (May 2005). *Nature reviews. Immunology* **5** (5): 375–386.
3. Paolicelli, D. (2009). *Biologics: Targets & Therapy* **3**: 369–376.

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

Canine IFNb 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 Canine IFNb 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.

Canine IFNb Standard (3 vials) – Each of the lyophilized Canine IFNb Standard 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.

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% Canine serum albumin in PBS, pH 7.3.
4. **Streptavidin-Horseradish Peroxidase (SA-HRP) Conjugate**
5. **Substrate Solution**, mixture of H_2O_2 and tetramethylbenzidine.
6. **Stop Solution**, 0.25 M H_2SO_4.
7. **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 300 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. All procedures are conducted at room temperature and remove bubbles for all steps. 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 **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 SA-HRP 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 up to 30 minutes till dark blue develops in high standard. 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. If high density is expected, plasma or serum sample should be diluted with equal volume of 1 x Assay Buffer 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 IFNb 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 Canine IFNb 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 canine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP4, IL-2, IL-5, IL-8, TGFβ1, TLR3, TNF-α.

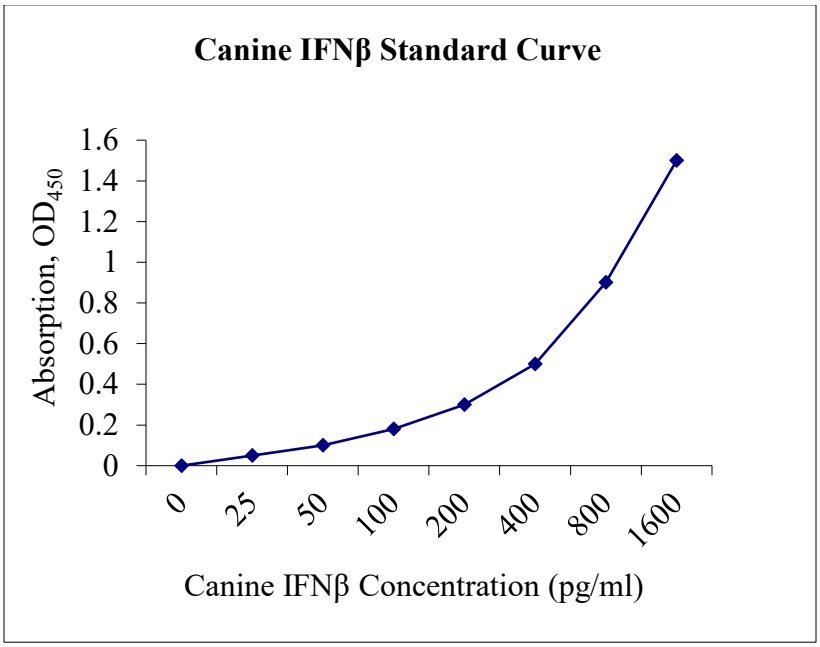
Calibration

This kit is calibrated against Canine IFNb.

Detection Range 25-1600 pg/ml

Assay Sensitivity 5 pg/ml

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



Related products
Canine IFNγ standard
Canine IFNγ detection antibody

DECLARATION

THIS REAGENT IS FOR IN VITRO LABORATORY TESTING AND RESEARCH USE ONLY. DO NOT USE IT FOR CLINICAL DIAGNOSTICS. DO NOT USE OR INJECT IT IN HUMANS AND ANIMALS.

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN HUMANS AND ANIMALS

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Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	• Inaccurate pipetting • Insufficient vortexing • OD_{450} too high for the high standard point • Air bubbles in wells.	• Check pipettes and ensure **equal dispensing**. • Vortex 30 sec for each of standard dilution steps and **vortex again (10 sec) before pipetting to the 96-well plate**. • Reduce substrate incubation time • Remove air bubbles in wells by pipette tip.
Low signal	• Improper preparation of reagents and storage • Too brief incubation times • Inadequate reagent volume or improper dilution • Standard defect	• Briefly spin down vials before opening. Reconstitute the powder thoroughly. Proper storage of plate and strip and detection antibody after first usage as shown in the datasheet. • Ensure sufficient incubation time including substrate incubation. Increase sample incubation to 2 hours. • Change a Standard vial.
Overflow in the standards	• Substrate incubation too long • Air bubbles in wells	• Observe the color development every 1-2 mins and reduce substrate incubation time. • Remove air bubbles in wells • Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells.
Large CV	• Inaccurate pipetting and mixing • Improper standard/sample dilutions. • Air bubbles in wells.	• Check pipettes and ensure accurate pipetting and thorough mixing and **equal dispensing**. • Use the correct dilution buffers • Remove air bubbles in wells by pipette tip.
High background	• Reagent reservoir issue • Plate is insufficiently washed and air bubbles in wells. • Contaminated wash buffer • Pipet tip contaminated	• Use a new reagent reservoir for Substrate Solution. • Increase wash to 4 times before adding substrate and ensure plate washer functions normally. Remove air bubbles in wells by pipette tip. • Make fresh wash buffer and wash thoroughly. • Use new pipette tips for blank wells.
No signal detected	• The procedure was misconducted. • Failures of spin down the contents in Detection Antibody and Standards. • Failure of Substrate or HRP • Samples overdiluted	• Ensure the step-by-step protocol. Spin vials of Detection antibody and Standard to complete recover the content. • Mix 100 µl of Substrate with 0.5 µl HRP and deep blue color should develop in 2 min. • Try a new standard vial and use positive control. • Try not dilute samples
Low sensitivity	• Improper dilutions of standards • Improper storage of the ELISA kit	• Ensure accurate and thorough dilutions of standards at each step. • Store detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate solution protected from light.