Nori® Guinea Pig IFNα ELISA 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 IFNα 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. Agents that activate the immune system, such as small imidazoquinoline molecules that activate TLR7, can induce IFN-α. Patients with recurrent melanomas receive recombinant IFN-α2b.[3] IFN-α 2b is also being used for treatment of ocular surface squamous neoplasia (OSSN). Both hepatitis B and hepatitis C are treated with IFN-α, often in combination with other antiviral drugs.[4]

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. Hauschild, A. et al. (2008). *Cancer* **112** (5): 982–994.
4. Cooksley, W. G. (Mar 2004). *MedGenMed : Medscape general medicine* **6** (1): 16.

PRINCIPLE OF THE ASSAY

This kit is for quantification of IFNα in dog. 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 Guinea Pig IFNα has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFNα present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Guinea Pig IFNα 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 IFNα 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 6 months.

Genorise

Nori® Guinea Pig IFNα ELISA Kit- DataSheet

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Assay Buffer	1	Stop Solution	1
HRP Conjugate	1	Reagent Diluent	1	Datasheet/Manual	1
Standard	3	MSDS/CoA	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

Guinea Pig IFNα 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. The vial contains sufficient Detection Antibody for a 96-well plate. Centrifuge 1 min at 6000 x g prior to open the vial. Add 200 µL of 1 x Reagent Diluent, vortex 30 sec and allow it to sit for 5 min prior to use. Take the entire 200 µL of detection antibody to 10.5 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.

Guinea Pig IFNα Standard (3 vials) – The lyophilized Guinea Pig IFNα Standard has a total of 4 vials. Each vial contains the standard sufficient for a 96-well plate. The non-reconstituted standard can be stored at 4°C ~ -20°C 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. Add 500 µL of 1 x Assay Buffer to a Standard vial to make the high standard concentration of 1,000 pg/ml. **Vortex 1 min** and allow it to sit for 5 min prior to use. A seven-point standard curve is generated using 2-fold serial dilutions in 1 x Assay Buffer, **vortex 30 sec** for each of dilution steps.

HRP Conjugate (53 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient HRP Conjugate for a 96-well plate. If the volume is less than 53 µL, add reagent diluent to reach 53 µL. Make 1:200 dilution in Reagent Diluent. If an entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of Reagent Diluent and vortex 30 sec prior to the assay. The rest of undiluted HRP Conjugate can be stored at 2 - 8° C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 25 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Assay Buffer, 20 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
Reagent Diluent, 21 mL.
Substrate Solution, 10.5 mL.
Stop Solution, 5.5 mL.

Assay Procedure

1. Lift the plate cover from the top left and cover the wells that are not used. **Vortex the standards and samples for 10 sec** before applying to the plate. Add 100 μL of **diluted sample** (see below) 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. Attention: **MUST vortex standards and samples for 10 sec before pipetting to the wells!**
2. Aspirate each well and wash with **1 x Assay Buffer**, repeating the process one time for a total of two washes. Wash by filling each well with 1 x 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 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 and observe the color development every 1-2 mins. Incubate for up to 20 minutes (**depending on signal. Stop** the reaction when it turns to dark blue in the highest standard wells) at room temperature. Over-incubation of the substrate will result in overflow of high standard and thus should be avoided. Avoid placing the plate in direct light.
8. When it gets to dark blue in the highest concentration of standard wells, add 50 μL of **Stop Solution** to each well to stop the reaction. 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.

Sample dilution: Samples should be diluted with four volumes 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.

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 standards at each of dilution steps is critical to acquire a normal standard curve and **vortex again (10 sec) before pipetting to the 96-well plate**.
3. HRP Conjugate 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.
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.

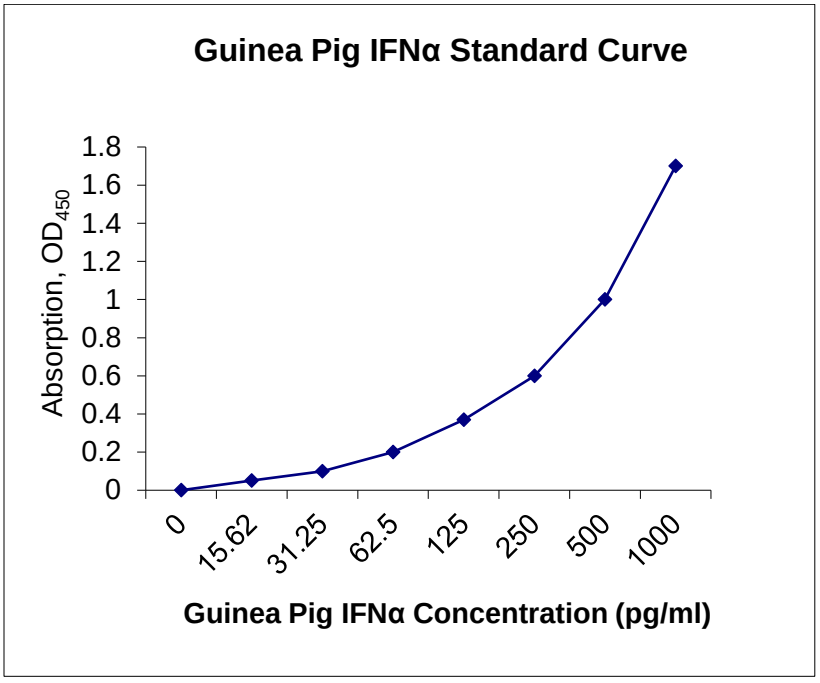
Calculation of Results

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 IFNα 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 Guinea Pig IFNα 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.



Specificity

The following recombinant Guinea Pig proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP4, HGF, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, MMP-2, MMP-9, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, sIL2R, sIL6R, VEGF.

Calibration

This kit is calibrated against a highly purified yeast-expressed recombinant Guinea Pig IFNα.

Detection Range

15-1000 pg/ml

Assay Sensitivity

3 pg/ml

Assay Precision

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

Sample types: Plasma, serum, cell/tissue lysates, cell culture supernatant, synovial fluid (SF), bronchoalveolar lavage (BAF), cerebrospinal fluid (CSF), urine, other biological fluid.

Related products

Guinea Pig IFNα Standard
Guinea Pig IFNα 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. WE ARE NOT RESPONSIBLE FOR ANY CONSEQUENCE OF USING IT IN HUMANS AND ANIMALS.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS AND ANIMALS

Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard curve • OD_{450} too high for the high standard point	• Check pipettes • Check and use the correct dilution buffer • 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
Low signal	• Improper preparation of standard, samples, detection antibody, and/or HRP 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 including substrate incubation. • Check pipettes and ensure correct preparation.
Overflow in the standards	• Substrate incubation too long	• Reduce substrate incubation time. • Observe the color development every 1-2 mins. • 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 thorough mixing. • Use the correct dilution buffers • Remove bubbles in wells.
High background	• Plate is insufficiently washed. • Contaminated wash buffer	• Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed. • 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 • 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