Nori® Equine 3-Plex ELISA Kit-IL6-IL8-TNFA DataSheet

PRINCIPLE OF THE ASSAY

This ELISA kit is for 3-Plex quantification of IL-1β, IL-8 and TNF-α in Equine samples. 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. Antibodies specific for Equine IL-1β (Columns 1-4), IL-8 (Columns 5-8) and TNF-α (Columns 9-12) have been pre-coated onto a microplate. Standards and samples are pipetted into the corresponding wells and any analytes of interest present are bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for the analyte of interest is added to the corresponding 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 analyte 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 kit at 4°C. The kit can be used in 3 months.

Plate map for antibody coating

IL-6: columns 1-4 (left, 4 columns, 32 wells).
IL-8: columns 5-8 (middle, 4 columns, 32 wells)
TNF-α: columns 9-12 (right, 4 columns, 32 wells)

Nori® Equine 3-Plex ELISA Kit-IL6-IL8-TNFA DataSheet

MATERIALS PROVIDED

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

Bring all reagents to room temperature before use.

Reagent Preparations

Equine 3-Plex Detection Antibody (3 vials) – The lyophilized Detection Antibody should be stored at 4°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. Each vial contains sufficient Detection Antibody for 4 columns (4 x 8 wells) in a 96-well plate. Add 200 µL of sterile 1 x PBS and vortex 30 sec. If all the 32 wells are used, take 200 µL of detection antibody to 3.3 mL of Reagent Diluent to make **Working dilution of detection antibody** and vortex 30 sec prior to the assay. If the partial antibody is used store the rest at -20°C until use.

Equine 3-Plex Standard (3 vials) – The lyophilized Equine 3-Plex Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve for one analyte. The unreconstituted standard can be stored at 4°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 tube. Add 500 µL of 1 x Assay Buffer to make the high standard concentration as indicated and **vortex for 1 min**. A seven-point standard curve is generated using 2-fold serial dilutions in the Assay Buffer, **vortex 30 sec** for each of dilution step.

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 53 µL HRP Conjugate sufficient for one 96-well plate. If the volume is less than 53 µL, add sterile 1 x PBS to reach 53 µL and vortex 10 sec. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of Reagent Diluent to make **working dilution of HRP Conjugate** and vortex 30 sec prior to the assay. The rest of undiluted HRP Conjugate 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 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 samples for 10 sec** prior to the assay. 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 corresponding 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. 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.

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 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 standard curve should be generated using a computer generated 4-PL curve-fit. The correlation coefficient (r^2) should be 0.990-1.000.

Specificity

The following recombinant Equine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.

ApoAI, BMP1, BMP2, BMP3, BMP4, BMP7, CRP, HGF, HSP27, IL-1 RI, IL-1RA, IL-2, IL-4, IL-5, IL-1b, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-21, IL-23, IFNγ, MMP-2, sIL-2R, sIL-6R, PDGF, PLA2G7, prolactin, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF RI, TNF RII, VEGF.

.

Calibration

This kit is calibrated against highly purified *E coli*-expressed recombinant Equine proteins of interest.

Detection Range

IL-6: 81-5200 pg/ml
IL-8: 10-600 pg/ml
TNF-α: 31-2000 pg/ml

Assay Sensitivity

IL-6: 16 pg/ml
IL-8: 2 pg/ml
TNF-α: 6 pg/ml

Assay Precision

IL-6: 7% (Intra-Assay), 9% (Inter-Assay)
IL-8: 5% (Intra-Assay), 11% (Inter-Assay)
TNF-α: 9% (Intra-Assay), 12% (Inter-Assay)

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

For Research Use Only

Related products

20 x Sample Diluent, GR103058
20 x PBS, Cat. GR103004-20
10 x ELISA Wash Buffer, Cat. GR103028
10 x ELISA Reagent Diluent, Cat. GR103055
Universal Blocking Buffer, Cat. GR103005
2 x Recombinant Protein Stabilizer, Cat. GR03014-2
5 x Recombinant Protein Stabilizer, Cat. GR103014-5
ELISA G-Blue Substrate Solution, Cat. GR103021

Plate maps

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
B	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
C	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
D	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
E	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
F	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
G	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα
H	IL-6	IL-6	IL-6	IL-6	IL-8	IL-8	IL-8	IL-8	TNFα	TNFα	TNFα	TNFα

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.
96-well 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 IL6-IL8-TNFA 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