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Nori® Equine Beta-Defensin 3 (BD-3) ELISA Kit-2 Plates DataSheet

Beta defensins are a family of mammalian defensins. The beta defensins are antimicrobial peptides implicated in the resistance of epithelial surfaces to microbial colonization. Defensins are 2-6 kDa, cationic, microbicidal peptides active against many Gram-negative and Gram-positive bacteria, fungi, and enveloped viruses,[1] containing three pairs of intramolecular disulfide bonds. On the basis of their size and pattern of disulfide bonding, mammalian defensins are classified into alpha, beta and theta categories. Every mammalian species explored thus far has beta-defensins. In cows, as many as 13 beta-defensins exist in neutrophils. However, in other species, beta-defensins are more often produced by epithelial cells lining various organs (e.g. the epidermis, bronchial tree and genitourinary tract). Beta-defensins induce the activation and degranulation of mast cells, resulting in the release of histamine and prostaglandin D2.[2] β-defensins are coding for genes which impact the function of the innate immune system.[3] These genes are responsible for production of antimicrobial peptides found in white blood cells such as macrophages, granulocytes and NK-cells, β-defensins are also found in epithelial cells.[4] β-defensins are cationic and can therefore interact with the membrane of invading microbes, which are negative due to lipopolysaccharides (LPS) and lipoteichoic acid (LTA) found in the cell membrane.[1] Due to changes in the electric potential, peptides will pass across the membrane and thus aggregate into dimers.[5] Pore complex will be created as a result of breaking the hydrogen bonds between the amino acids in the terminal end of the strands connecting defensins monomers.[7] Formation of pore complex will cause membrane depolarization and cell lysis.[5] Defensins not only have the ability to strengthen the innate immune system but can also enhance the adaptive immune system by chemotaxis of monocytes, T-lymphocytes, dendritic cells and mast cells to the infection site.[5] Defensins will also improve the capacity of macrophage phagocytosis.[5]

References

1. White SH, et al. (1995). *Curr. Opin. Struct. Biol.* **5** (4): 521–7.
2. Bensch KW, et al. (1995). *FEBS Lett.* **368** (2): 331–5.
3. Hellgren O, Sheldon BC (2011). *Molecular Ecology Resources* **11** (4): 686–692.
4. Ganz T (2003). *Nat. Rev. Immunol.* **3** (9): 710–20.
5. van Dijk A, et al. (2008). *Vet. Immunol. Immunopathol.* **124** (1-2): 1–18.
6. Sugiarto H, Yu PL (2004). *Biochem. Biophys. Res. Commun.* **323** (3): 721–7.

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-linked immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for beta-defensin 3 (BD-3) has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BD-3 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for BD-3 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 BD-3 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

Storage

Store 4 °C. The kit can be used in 6 months.

This package insert must be read in its entirety before using this product.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	2	20 x PBS	2	Substrate Solution	1
Detection Antibody	2	80 x Assay Buffer	1	Stop Solution	1
HRP Conjugate	1	MSDS	1	DataSheet	1
Standard	4			96-well plate sheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

Equine beta-Defensin 3 Detection Antibody (2 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 a 96-well plate. Add 200 µL of sterile 1 x PBS and vortex 30 sec. If the entire 96-well plate is used, take 200 µL of detection antibody to 10.5 mL of 1 x PBS 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 beta-Defensin 3 Standard (4 vials) – Each of the lyophilized Equine beta-Defensin 3 Standard vial contains the standard sufficient for generating a standard curve. 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 of 1000 pg/ml 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 (110 µl) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 110 µL HRP Conjugate sufficient for two 96-well plates. If the volume is less than 110 µL, add sterile 1 x PBS to reach 110 µL and vortex 10 sec. Make 1:200 dilutions in 1 x PBS. If the entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of 1 x PBS 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, 2 x 25 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
80 x Assay Buffer, 100 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
Substrate Solution, 21 mL.
Stop Solution, 11 mL.

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

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Assay Procedure

1. All procedures are conducted at room temperature (20-25 ºC) and ensure **equal pipetting/dispensing** at each step and remove air bubbles in the wells for all steps.
2. Lift the plate cover and cover the unused wells or reseal the unused strips in the aluminum bag with desiccant at 4 ºC. **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 for **2 hours**. Attention: **MUST vortex standards and samples for 10 sec before pipetting to the wells!**
3. Aspirate each well and wash with 300 µL of **1 x Assay Buffer** for two times. Wash by filling each well with 1 x Assay Buffer 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.
4. Add 100 µL of the **working dilution of Detection Antibody** to each well. Cover the plate and incubate for 1 hour.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µL of the **working dilution of HRP Conjugate** to each well. Cover the plate and incubate for 20 minutes. Avoid placing the plate in direct light.
7. Repeat the aspiration/wash as in step 3 but **wash 4 times** instead.
8. Add 100 µL of **Substrate Solution** to each well and observe the color development every 1-2 mins. Incubate for up to **30 minutes** (**depending on signal. Stop** the reaction when it turns to dark blue in the highest standard wells). Over-incubation of the substrate will result in overflow of high standard and thus should be avoided. Avoid placing the plate in direct light.
9. 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.
10. 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: If high density is expected, samples should be diluted with one 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.

Precaution and Technical Notes

1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected and make sure no air bubbles in wells before adding reagents.
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 performance of the assay.

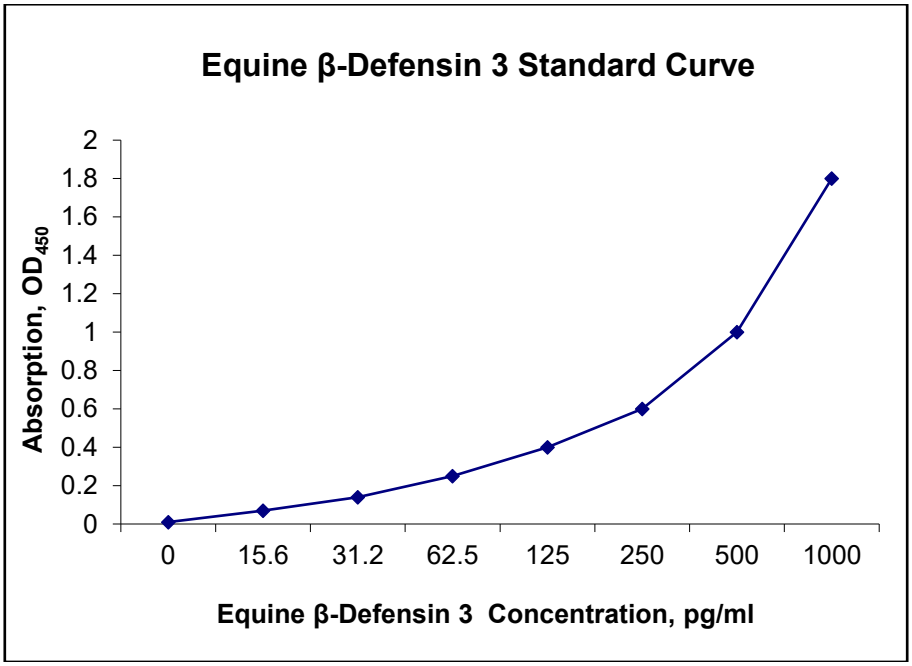
Calculation of Results

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 BD-3 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 equine beta-defensin 3 (BD-3) 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 equine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
Adiponectin, ApoAI, BMP7, CCL5, CRP, FGF acidic, HGF, HSP27, IGF-1, IL-1α, IL-1β, IFN-α, IFN-β, IFNγ, MMP-9, PDGF, PLA2G7, prolactin, TLR4, TGF-β1, TNF-α, VEGF, VEGF-R1.

Calibration

This kit is calibrated against equine beta-defensin 3 (BD-3)

Detection Range

15-1000 pg/ml

Assay Sensitivity

1.8 pg/ml

Assay Precision

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

Related products

Recombinant equine beta-defensin 3 (BD-3)
Equine beta-defensin 3 (BD-3) 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.