Nori® Canine MMP-2 ELISA Kit DataSheet

Matrix metalloproteinase-2 (MMP-2) is known as 72 kDa type IV collagenase, and gelatinase A is an enzyme that in humans is encoded by the *MMP2* gene.(1) Proteins of the MMP family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. This gene encodes an enzyme which degrades type IV collagen, the major structural component of basement membranes. The enzyme plays a role in endometrial menstrual breakdown, regulation of vascularization and the inflammatory response.

Activation of MMP-2 requires a proteolytic processing. A complex of membrane type 1 MMP (MT1-MMP/MMP14) and tissue inhibitor of metalloproteinase 2 recruits pro-MMP 2 from extracellular milie to the cell surface. Activation then requires active molecule of MT1-MMP and auto catalytic cleavage. Clustering of integrin chain promotes activation of MMP-2. Another factor that will support the activation of MMP-2 is cell-cell clustering. A wild-type activated leukocyte cell adhesion molecule (ALCAM) also required to activate MMP-2. Mutations in the MMP2 gene are associated with Torg-Winchester syndrome, multicentric osteolysis and arthritis syndrome.(2) MMP2 has been shown to interact with THBS2,(3) TIMP2,(4-7) Thrombospondin 1,(4) CCL7 (8) and TIMP4.(6, 7)

Reference

1. Devarajan P, et al. *J. Biol. Chem.* **267** (35): 25228–32.
2. Martignetti JA, et al. *Nat. Genet.* **28** (3): 261–5.
3. Bein K, Simons M. *J. Biol. Chem.* **275** (41): 32167–73.
4. Morgunova E, et al. *Proc. Natl. Acad. Sci. U.S.A.* **99** (11): 7414–9.
5. Overall CM, et al. *J. Biol. Chem.* **275** (50): 39497–506.
6. Bigg HF, et al. *J. Biol. Chem.* **272** (24): 15496–500.
7. Kai HS, et al. *J. Biol. Chem.* **277** (50): 48696–707.
8. McQuibban GA, et al. *Science* **289** (5482): 1202–6.

PRINCIPLE OF THE ASSAY

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

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	10 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet/Manual	1
Standard	3	20 x Standard/Sample Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

Reagent Preparations

Canine MMP-2 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 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of sterile 1 x PBS to the vial and vortex for 30 sec. Take the 200 µL of detection antibody to 9.8 mL of 1 x Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Canine MMP-2 Standard (3 vials) – The lyophilized Canine MMP-2 Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a calibration curve. The unreconstituted standard can be stored at -20° C for up to 3 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 Standard/Sample Diluent to a Standard vial to make the high standard concentration of 16 ng /ml. Vortex 30 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in the Standard Diluent, vortex 30 sec for each of dilution step.

Detection Agent (1 vial) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. The vial contains sufficient detection agent for a 96-well plate. add 200 µL of sterile 1 x PBS to a vial and vortex 30 sec. Make 1:50 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 200 µL of Detection Agent to 9.8 mL of the Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 3 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 Wash Buffer, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent– Add 3 mL of sterile 1 x PBS to make 10 x Reagent Diluent, vortex 1 min and allow it to sit for 15 min to completely dissolve. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS and mix well.
20 x Standard/Sample Diluent, 10 mL Dilute to 1 x Standard/Sample Diluent with 1 x PBS prior to use.
Substrate Solution, 10 mL
Stop Solution, 5 mL.

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Assay Procedure

1. Lift the plate cover and cover the wells that are not used. Vortex briefly the samples prior to the assay. Add 100 µL of sample (such as plasma or serum) or standard to each well, and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash 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 Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the 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 Detection Agent 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 5-10 minutes 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

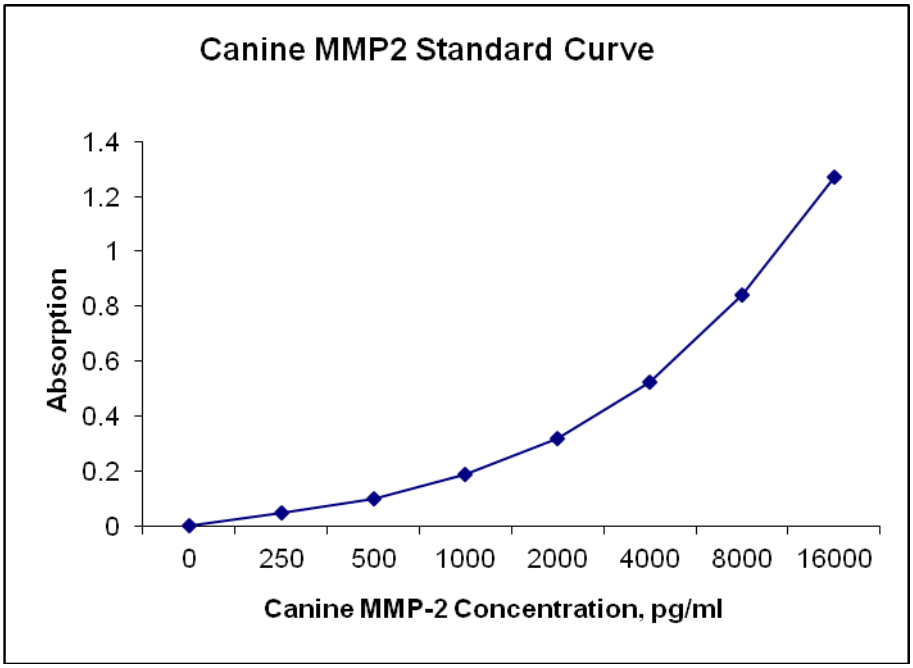
1. Thorough mixing of the standard dilution in each step by vortex is critical to ensure the good standard curve.
2. If MMP-2 exceeds the upper limit of the detection, the sample needs to be diluted with 1 x Wash Buffer. The dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. A standard curve should be generated for each set of samples assayed.
6. This kit should not be used beyond the expiration date on the label.
7. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
8. Use a fresh reagent reservoir and pipette tips for each step.
9. It is recommended that all standards and samples be assayed in duplicate.
10. 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 MMP-2 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 MMP-2 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.
BMP1, BMP2, BMP4, HGF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFN-γ, TGFβ1, TGFβ2, TGFβ3,TLR1, TLR2, TLR3, TNF-α, VEGF

Calibration

This kit is calibrated against a highly purified yeast-expressed recombinant canine MMP-2.

Detection Range

250-16000 pg/ml

Assay Sensitivity

100 pg/ml

Assay Precision

Intra-Assay %CV: 5; Inter-Assay %CV: 9

For Research Use Only

Related products

10 x ELISA Wash Buffer, GR103014
10 x Reagent Diluent, GR103028
20 x PBS, 103004-20
ELISA Substrate, GR103021
ELISA Stop Solution, GR103055
ELISA Detection Agent, GR103044
Canine MMP-2 standard
Canine MMP-2 detection antibody