



Nori® Canine IgA ELISA Kit-2 Plates DataSheet

Immunoglobulin A (IgA) is an antibody that plays a critical role in mucosal immunity. More IgA is produced in mucosal linings than all other types of antibody combined;^[1] between three and five grams are secreted into the intestinal lumen each day.^[2] This accumulates to 75% of the total immunoglobulin produced in the entire body.^[3] IgA has two subclasses (IgA1 and IgA2) and can exist in a dimeric form called *secretory IgA* (sIgA). In its secretory form, IgA is the main immunoglobulin found in mucous secretions, including tears, saliva, colostrum and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium. It is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh gastrointestinal tract environment and provide protection against microbes that multiply in body secretions. IgA is a poor activator of the complement system, and opsonises only weakly. Its heavy chains are of the type α . In the blood, IgA interacts with an Fc receptor called Fc α RI (or CD89), which is expressed on immune effector cells, to initiate inflammatory reactions.^[4] Ligation of Fc α RI by IgA containing immune complexes causes antibody-dependent cell-mediated cytotoxicity (ADCC), degranulation of eosinophils and basophils, phagocytosis by monocytes, macrophages, and neutrophils, and triggering of respiratory burst activity by polymorphonuclear leukocytes.^[4] Polymeric IgA (mainly the secretory dimer) is produced by plasma cells in the lamina propria adjacent to mucosal surfaces. It binds to the polymeric immunoglobulin receptor on the basolateral surface of epithelial cells, and is taken up into the cell via endocytosis. The receptor-IgA complex passes through the cellular compartments before being secreted on the luminal surface of the epithelial cells, still attached to the receptor. Proteolysis of the receptor occurs, and the dimeric IgA molecule, along with a portion of the receptor known as the secretory component, are free to diffuse throughout the lumen.^[5] In the gut, it can bind to the mucus layer on top of the epithelial cells to form a barrier capable of neutralizing threats before they reach the cells. Decreased or absent IgA, termed selective IgA deficiency, can be a clinically significant immunodeficiency. *Neisseria gonorrhoeae* (which causes gonorrhoea), *Streptococcus pneumoniae*, and *Haemophilus influenzae* type B all releases a protease which destroys IgA.

Reference

1. S Fagarasan and T Honjo (2003). *Nat. Rev. Immunology* **3** (1): 63–72.
2. P. Brandtzaeg, R. Pabst (2004). *Trends Immunology* **25** (11): 570–577.
3. AJ Macpherson and E Slack. (2007). *Curr Opin Gastroenterol.* **23** (6): 673–678.
4. CS Kaetzel et al. (1991). *Vet. Res.* **37** (3): 455–67.
5. CS Kaetzel et al. (1991) *Proc Natl Acad Sci USA* **88** (19): 8796–8800.

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 and the sensitivity of the assays. The sensitivity is 125-folds higher than the conventional method. An antibody specific for Canine IgA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgA present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Canine IgA 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 IgA 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	2	20 x PBS	2	Substrate Solution	1
Detection Antibody	2	80 x Assay Buffer	1	Stop Solution	1
HRP Conjugate	1	96-well plate sheet	2	DataSheet/Manual	1
Standard	4				

Bring all reagents to room temperature before use.

Reagent Preparations

Canine IgA Detection Antibody (2 vials) – The lyophilized Detection Antibody should be stored at 4°C or -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. Each vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of 1 x PBS to the antibody vial and vortex 15 sec and allow it to sit 5 min. Take 200 µL of the detection antibody to 10.5 mL of 1 x PBS to make **working dilution of Detection Antibody** and vortex 30 sec if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Canine IgA Standard (4 vials) – Each lyophilized Canine IgA Standard vial contains the standard sufficient for generating a calibration curve. The unreconstituted standard can be stored at 4°C or -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 tube. Add 500 µL of 1 x Assay Buffer to a standard vial to make the high standard concentration of 6 ng/ml, **vortex for 1 min** and allow it to sit for 5 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 briefly. Make 1:200 dilution in 1 x PBS. If the entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of the 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 2 - 8°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, 10 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.

Substrate Solution, 21 mL.

Stop Solution, 11 mL.



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Assay Procedure

1. All procedures are conducted at room temperature (20-25 °C) and ensure **equal pipetting/dispensing** at each step and remove air bubbles in the wells for all steps.
2. Lift the plate cover from the top left and cover the wells that are not used. For strip plate, store the unused strips in the sealed foil bag at 4 °C. **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**.
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 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 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.



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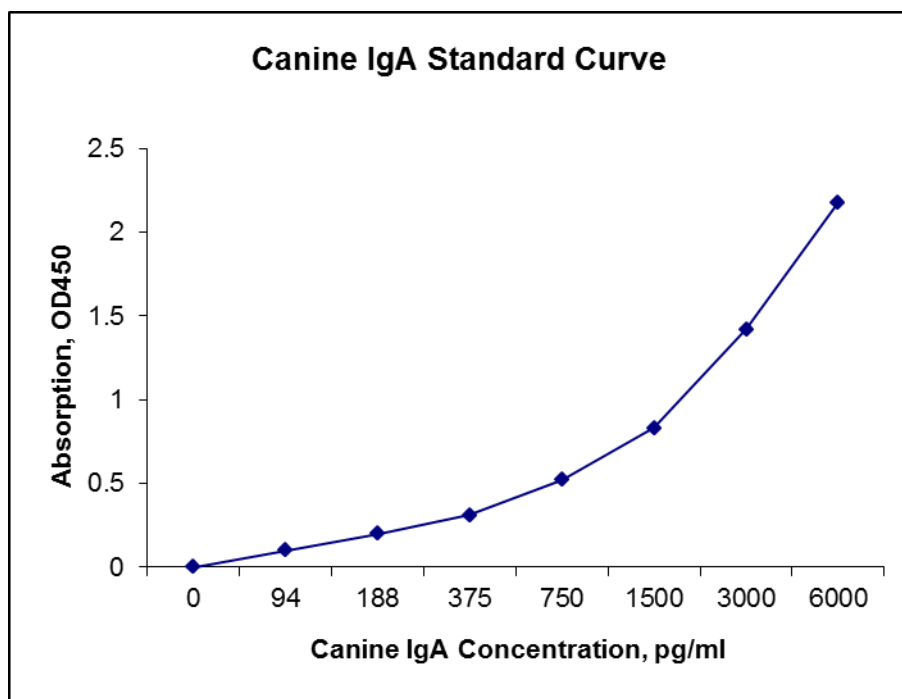
Calculation of Results

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 IgA 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 IgA 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.





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Specificity

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

BMP4, IL-1 β , IFN γ , TGF β 1, TLR3, TNF- α , VEGF.

Calibration

This kit is calibrated against a highly purified canine IgA.

Detection Range

94-6000 pg/ml

Assay Sensitivity

18 pg/ml

Assay Precision

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

Related products

Canine IgA Standard

Canine IgA detection antibody

Related products

Bovine IgA Standard

Bovine IgA 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	<ul style="list-style-type: none"> Inaccurate pipetting Insufficient vortexing OD₄₅₀ too high for the high standard point Air bubbles in wells. 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Improper preparation of reagents and storage Too brief incubation times Inadequate reagent volume or improper dilution Standard defect 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Substrate incubation too long Air bubbles in wells 	<ul style="list-style-type: none"> Observe the color development every 1-2 mins and reduce substrate incubation time. Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells. Remove air bubbles in wells
Large CV	<ul style="list-style-type: none"> Inaccurate pipetting and mixing Improper standard/sample dilutions. Air bubbles in wells. 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Reagent reservoir issue Plate is insufficiently washed and air bubbles in wells. Contaminated wash buffer Pipet tip contaminated 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> The procedure was misconducted. Failures of spin down the contents in Detection Antibody and Standards. Failure of Substrate or HRP Samples overdiluted 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Improper dilutions of standards Improper storage of the ELISA kit 	<ul style="list-style-type: none"> 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.