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User's Manual

Canine CD200 ELISA Kit

REF **DEIASL537**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This ELISA kit is for quantification of CD200 in Canine 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.

General Description

Cluster of Differentiation 200 (CD200) also known as OX-2 membrane glycoprotein and is encoded by the CD200 gene. [1] CD200 is a type-1 membrane glycoprotein, which contains two immunoglobulin domains, and thus belongs to the immunoglobulin superfamily. Studies of the related genes in mouse and rat suggest that this gene may regulate myeloid cell activity and delivers an inhibitory signal for the macrophage lineage in diverse tissues. Multiple alternatively spliced transcript variants that encode different isoforms have been found for this gene. CD200 interacts with its receptor CD200R to transmit signals and aberrant expression of CD200/CD200R might play a role in psoriasis vulgaris pathophysiology and disease severity. [2] CD200 is overexpressed in neuroblastoma and regulates tumor immune microenvironment. [3] CD200 down regulates macrophage lineage and basophil function. [4] [5]

Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance and the sensitivity of the assays. An antibody specific for Canine CD200 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD200 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Canine CD200 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 CD200 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

Reagents And Materials Provided

1. Antibody Precoated Plate: 1
2. Detection Antibody: 1
3. HRP Conjugate: 1
4. Standard: 3
5. 20 x PBS: 1
6. 20 x Assay Buffer: 1
7. Reagent Diluent: 1
8. Substrate Solution: 1
9. Stop Solution: 1

Bring all reagents to room temperature before use.

Storage

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

Specimen Collection And Preparation

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

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.

Reagent Preparation

Canine CD200 Detection Antibody (1 vial) – 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. The 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 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.

Canine CD200 Standard (3 vials) – The lyophilized Canine CD200 Standard has a total of 3 vials. Each 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 8 ng/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 (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, 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 and remove air bubbles in the wells. 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. Remove bubbles in the wells.
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 and remove air bubbles in the wells.
5. Add 100 µL of the working dilution of HRP Conjugate to each well and remove air bubbles in wells. 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 and remove air bubbles in the wells.
7. Add 100 µL of Substrate Solution to each well and observe the color development every 1-2 mins and remove bubbles in the wells. 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 and remove bubbles in the 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.
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 and remove bubbles in the wells. 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.

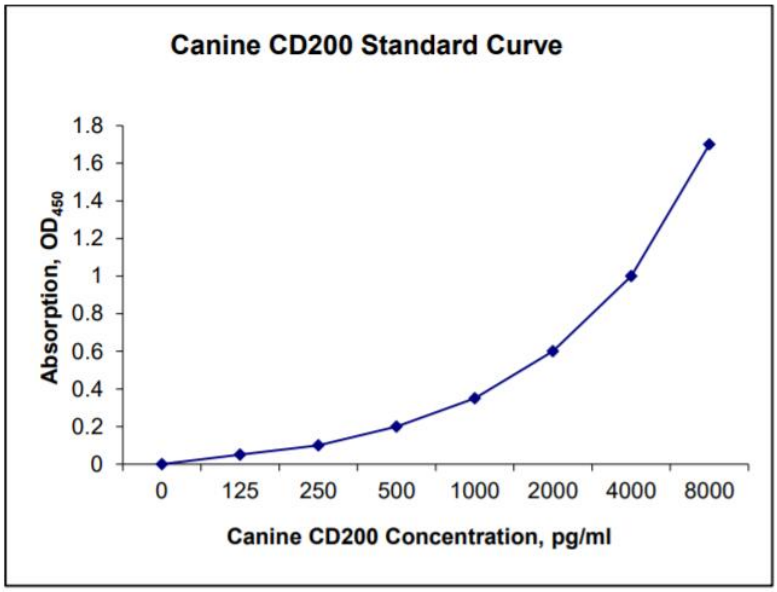
Calculation

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 CD200 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.

Typical Standard Curve

The graph below represents typical data generated when using this Canine CD200 ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark TM Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r2) is 0.999-1.000.



Precision

Intra-Assay %CV: 6;

Inter-Assay %CV: 9

Detection Range

125-8,000 pg/ml

Sensitivity

25 pg/ml

Specificity

The following recombinant Canine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference. ApoAI, BMP1, CRP, HGF, HSP27, IL-1α, IFN, MMP-2, PDGF, PLA2G7, prolactin, TGFβ1, TLR3, TNF-α, VEGF.

Precautions

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 sensitivity of the assay.

References

1. McCaughan,G.W., et al. (1987) Immunogenetics 25 (5), 329-335.
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4. Hoek RM, et al. (2000). Science. 290(5497): 1768–71.
5. Shiratori I, et al. (2005). J. Immunol. 175 (7): 4441–9.