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# Canine Toll Like Receptor 9(TLR-9) ELISA Kit

96 Tests

Catalogue Number:NSL0984Ca

Store all reagents at -20℃ ( 2-8℃~2 weeks )

Validity Period: 12 months

For samples:

In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY !

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS !

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

## Purpose

Our Canine Toll Like Receptor 9(TLR-9) ELISA Kit is to assay TLR-9 levels in Canine serum, plasma, culture media or any biological fluid.

## Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate has been pre-coated with an anti-TLR-9 monoclonal antibody and the detection antibody is a polyclonal antibody which be biotinylated in this kit. Samples and biotinylated polyclonal antibody are added to the appropriate Microelisa stripplate wells in sequence and washed out with TBS or PBS. Then HRP-Avidin is added to each Microelisa stripplate well. The TMB is used for coloration after the HRP-Avidin has been reacted thoroughly and washed out by TBS or PBS. The TMB will appear blue under peroxidase activity then turn yellow after the addition of the stop solution. The depth of color is positively correlated with the factors to be measured in the sample. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TLR-9. You can calculate the concentration of TLR-9 in the samples by comparing the OD of the samples to the standard curve.

**Note:** One antibody molecule can be labeled with multiple biotin molecules, and one biotin molecule can bind a horseradish peroxidase labeled avidin, thus bringing a large amount of horseradish peroxidase bound to the antibody, which has higher sensitivity and signal amplification effect than the traditional direct horseradish peroxidase labeled antibody.

## Materials provided with the kit

| | Materials provided with the kit | 96 Tests | 48 Tests | Storage |
|---|---|---|---|---|
| 1 | Microelisa stripplate | 8×12 | 8×6 | 2-8/-20℃ |
| 2 | Standard | 2 vial(lyophilized) | 1 vial(lyophilized) | 2-8/-20℃ |
| 3 | Biotinylated antibody (1:100) | 120ul | 60ul | 2-8/-20℃ |
| 4 | HRP-Avidin(1:100) | 130ul | 65ul | 2-8/-20℃ |
| 5 | HRP-Avidin diluent | 12ml | 6ml | 2-8/-20℃ |
| 6 | Antibody diluent | 12ml | 6ml | 2-8/-20℃ |
| 7 | Standard diluent | 15ml | 7.5ml | 2-8/-20℃ |

| | | | | |
|---|---|---|---|---|
| 8 | Sample diluent | 15ml | 7.5ml | 2-8/-20℃ |
| 9 | Washing buffer (1:25) | 20ml | 10ml | 2-8/-20℃ |
| 10 | Color Reagent A | 10ml(Avoid light) | 5ml(Avoid light) | 2-8/-20℃ |
| 11 | Color Reagent B | 1.5ml | 0.75ml | 2-8/-20℃ |
| 12 | Stop Solution | 10ml | 5ml | 2-8/-20℃ |
| 13 | Manual | 1 | 1 | RT |
| 14 | Closure plate membrane | 3 | 3 | RT |

## Notes:

1. The re-dissolved standard can't be stored again once be prepared, so please do not to re-freeze it once it has been reconstituted. The kit should be stored at 2-8℃ before using.
2. Due to shaking/inversion during transport, centrifuging of the tubes/bottles of the kit might be necessary to consolidate the material contained within. Tubes should be shaken manually or centrifuged for 1 min at 1000rpm to pool all material to the bottom.
3. Concentrated washing buffer might crystallize slightly. Use a water bath to help the dissolution during diluting process. The crystals must be totally dissolved when preparing the washing buffer.
4. The prepared standard is intended to only be a single-use aliquot, please do not try to re-use it that has already been tested. Please use the second vial provided if you run the assay again.Standards are always recommended to be tested in duplicate or triplicate
5. Do not mix batches/lots from other orders of this kit, or from different kits.
6. Ensure the reagents are well mixed. For the reagents in the plate, adequate mixing is particularly important for accurate test results. It is recommended to employ a micro-oscillator (at the lowest frequency). If a micro-oscillator is not available, please slightly shake the microplate manually for 1 min, in a circular motion in order to make sure the wells are sufficiently mixed.
7. The chromogen reagent is sensitive to light, therefore please avoid exposing to light.
8. Stop solution is 1M sulfuric acid, so please pay close attention to safety when it is used.
9. Sample addition should always be done via pipette or similar instrument. Calibrate the instrument prior to running the assay in order to avoid experimental errors. Please add samples to the wells quickly, as it is recommended to control the sample addition time to less than ~5 minutes. You might want to consider multi-pronged pipettes if this helps with the loading time.
10. New standard curves should be made for every new run of the assay. If the observed concentrations of test samples are too high (OD value of the sample is higher than that of standard well maximum concentration), dilute by a certain factor, and correct for said factor in the end calculations.
11. Samples containing NaN3 can't be tested due to NaN3 inhibiting the activities of horseradish peroxidase (HRP).
12. When washing plate via plate washer, the volume of buffer injected into each well should be slightly more than 350μl. Make sure the sampling head is not jammed or blocked. Also, if washing by hand, please take care when using an absorbent material to remove excess water – make sure this absorbent material wasn't used to clean any of the other

reagents to prevent contamination.

13. Read OD within 10 minutes after the coloration reaction termination by Stop solution.
14. If duplicate wells were performed, the mean value of the wells should be used.
15. Hemolyzed samples may cause false positive results, so we consider these samples to be incompatible with this kit.
16. During the assay, please try to control the humidity to ~60%.
17. We recommend regularly checking the thermostat and calibration in order to confirm the incubation temperature remains at a stable 37℃.
18. User should check the concentration of samples according relevant literature then decide to dilute the sample, so that the concentration of samples after be diluted can be in the best of detection range of Elisa kit. The dilution of the sample should documented in detail.

# Sample preparation

1. **Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.

2. **Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing.

Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

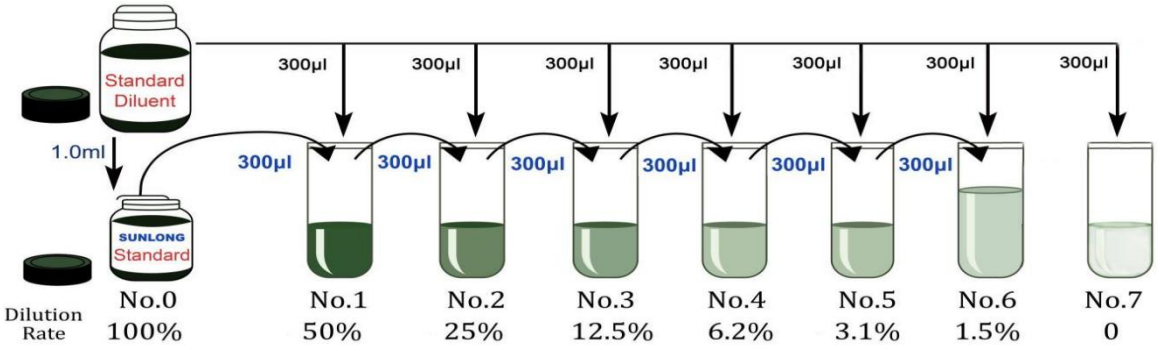
5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

# Test preparation

1. Please put Elisa kit in Room Temperature, wait and until it reaches room temperature before test.
2. Use double distilled water to dilute the concentrated wash buffer(1:25). Return unused wash buffer back to the box.
3. Standard: Add 1.0ml Standard Diluent to the vial of lyophilized standard and wait for 30 min. After the standard dissolved completely, mix it slightly and label No.0 on the tube. **Note:** Ensure the lyophilized standard dissolved and well mixed completely.
4. The dilution method of standard: Prepare 7 clean tubes and label No.1, 2, 3, 4, 5, 6, 7. Add 300μL Standard Diluent into each tube. Pipette out 300μl diluent from No.0 into No.1 and mix well. Then Pipette out 300μl diluent from No.1 into No.2 and mix well. Repeat these steps until to the tube of No.6. The tube of No.7 (0) as the negative control.It is recommended to use the following concentration values for the standard curve: 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml,0.312ng/ml.

| | | |
|---|---|---|
| 20ng/ml | Standard No.0 | lyophilized standard+1.0ml Standard Diluent |
| 10ng/ml | Standard No.1 | 300μl Standard No.0 + 300μl Standard diluent |
| 5ng/ml | Standard No.2 | 300μl Standard No.1 + 300μl Standard diluent |
| 2.5ng/ml | Standard No.3 | 300μl Standard No.2 + 300μl Standard diluent |
| 1.25ng/ml | Standard No.4 | 300μl Standard No.3 + 300μl Standard diluent |
| 0.625ng/ml | Standard No.5 | 300μl Standard No.4 + 300μl Standard diluent |
| 0.312ng/ml | Standard No.6 | 300μl Standard No.5 + 300μl Standard diluent |
| 0ng/ml | Standard No.7 | 300μl Standard diluent only(negative control) |



**Note**: The dissolved standard solution should be discarded after running the assay – it is not reusable.

5.Biotinylated Antibody: take the amount needed for the experiment, dilute with Antibody Diluent in a proportion of 1:100. This should be prepared 30min in advance, do not re-using for additional assaying.

6. HRP-Avidin: take the amount needed for the experiment, dilute with the HRP-Avidin Diluent in a proportion of 1:100. This should be prepared 30min in advance, do not re-using for additional assaying.

7. Color Reagent: mix 9 Color Reagent A and 1 Color Reagent B by the proportion of 9:1. This should be prepared 30min in advance.

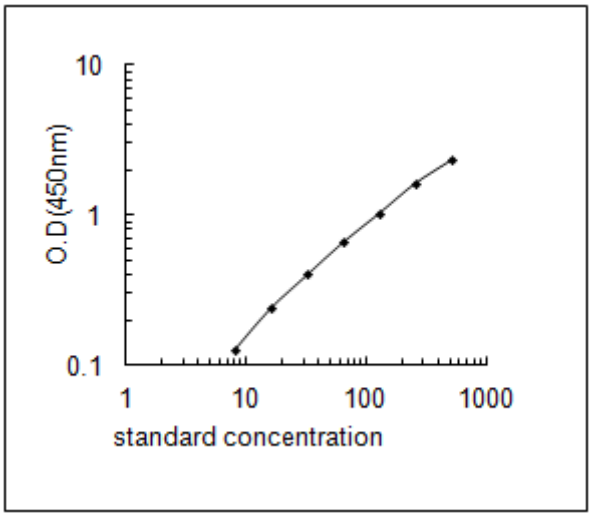
## Operation Steps

1. Take out the demand number of strips, wait and until it reaches room temperature. The unused strips and desiccant should be placed back and stored at 2-8℃.
2. Set reserved blank wells (if measuring at dual-wavelength, the blank wells can be ignored)
3. Add standards or samples to the corresponding wells (100μL for each well). Incubate 90 min at 37℃ after sealed with Closure plate membrane.
4. Prepare required quantity of Biotinylated Antibody 30min in advance.
5. Wash ELISA plate 2 times
6. Add prepared Biotinylated Antibody to each well (100μL per well). Incubate 60 min at 37℃ after sealed with Closure plate membrane.
7. Prepare required quantity of HRP-Avidin 30min in advance.
8. Wash ELISA plate 3 times
9. Add prepared HRP-Avidin to each well, please note don't add it to the blank wells (100μl for each). Incubate 30 min at37℃ after sealed with Closure plate membrane.
10. Wash ELISA plate 5 times.
11. Add 100μL of the prepared Color Reagent to individual wells include blank well, avoid light and incubate at 37℃. When the standard curve has a high concentration of dark color, there is a clear color gradient, it can be terminated. The time of chromogenic reaction should be controlled within 30 min.
12. Add 100μL Stop Solution to each well include the blank well. Mix well then Read OD （450nm）within 10 min.

## Calculation of Results

1. The OD values of each sample and standard should subtract the values of the blank well.
2. Draw standard curve manually. Take the concentration of standards as X- and OD value as Y-coordinates. Use a smooth line to connect each coordinate point of the standard values. The concentration of samples can be found by inputting the sample OD values into the line equation for the standard curve. It is recommended to employ professional curve software (e.g. curve expert 1.3) to analyze and compute the results.
3. If the sample OD is higher than the highest standard in standard curve, the sample should be diluted appropriately and the retest. Multiplied by the dilution rate when we calculate the concentration.

## Reference curve




This diagram is for reference only

## Assay range

20ng/ml-0.312ng/ml

Intra-Assay: CV<8%

Inter-Assay: CV<12%

Recovery: 70 - 110 percent

## Sensitivity

0.06ng/ml

## Storage and validity

1. Storage: -20℃.[Short-term should be stored at 2-8℃ ~2 weeks]
2. Validity: 12 months
3. Production Date: On the lable of elisa box