

# DOG NGAL ELISA

## Life Diagnostics, Inc., Catalog Number: NGAL-4

### INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL) is an acute phase protein of ~21 kDa. It binds iron-containing siderophores and has antibacterial properties. Plasma and urinary levels of NGAL are significantly increased in dogs with chronic kidney disease and acute kidney injury (ref 1).

### PRINCIPLE OF THE ASSAY

The assay uses two dog NGAL antibodies developed at Life Diagnostics; one for solid-phase immobilization (microtiter wells) and one, conjugated to horseradish peroxidase (HRP), for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in NGAL molecules being sandwiched between the immobilization and detection antibodies. The wells are washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If NGAL is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of NGAL is proportional to absorbance and is derived from a standard curve.

### MATERIALS AND COMPONENTS

#### Materials provided with the kit:

- NGAL antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- NGAL stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- Diluent; YD50-1, 2 x 50 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

#### Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Microcentrifuge tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

### STORAGE

The kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

### GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

### WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

### STANDARD PREPARATION

1. The NGAL stock is provided lyophilized. It consists of recombinant dog NGAL in a stabilizing protein matrix. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved (*the reconstituted standard remains stable for at least 1 day at room temperature but should be frozen at -20°C after reconstitution if future use is intended*).
2. Label eight microcentrifuge tubes as 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 ng/ml.
3. In the tube labeled 5 ng/ml prepare the 5 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 ng/ml.
5. Prepare the 2.5 ng/ml standard by mixing 250 µl of the 5 ng/ml standard with 250 µl of diluent in the tube labeled 2.5 ng/ml.
6. Similarly prepare the 1.25, 0.625, 0.313, 0.156 and 0.078 standards by two-fold serial dilution.

### SAMPLE PREPARATION

**Serum.** We found that NGAL is present in dog serum at concentrations from 5 to >140 ng/ml. Levels in serum from healthy animals were  $11 \pm 5.5$  ng/ml (mean  $\pm$  SD, n = 24). To obtain values within the range of the standard curve we suggest testing at a 40-fold dilution (optimal dilution factors should be determined by the end user). To avoid matrix effects do not test serum at dilutions less than 5-fold<sup>1</sup>.

**Urine.** We found NGAL levels of 0.8 ng/ml and 40 ng/ml in urine from healthy and diabetic dogs respectively. Urine should be diluted 2-fold or more prior to assay.

Use only the diluent provided with the kit for sample dilution.

### ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).

<sup>1</sup> Dog plasma has not yet been tested in the assay at Life Diagnostics. It is expected that the assay will work with plasma if samples are diluted to eliminate matrix effects.

5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

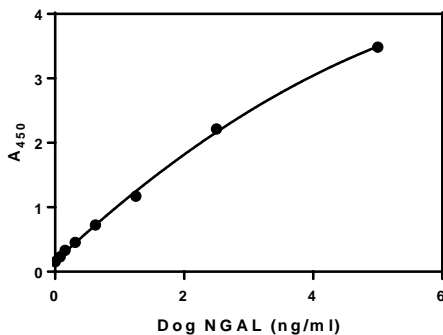
### CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the concentration.
2. Fit the standard curve to a second order polynomial (quadratic) equation.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum sample.
4. If the A<sub>450</sub> values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

### TYPICAL STANDARD CURVE

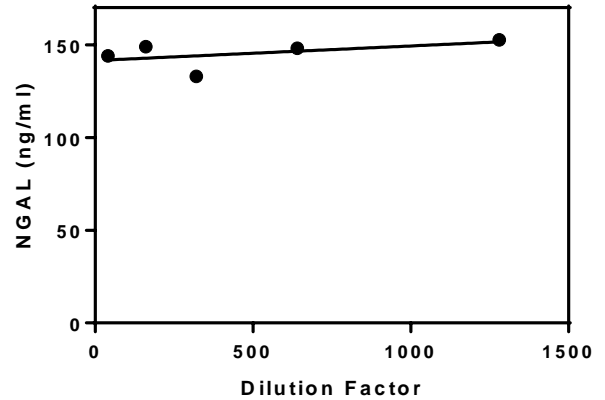
A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

NGAL (ng/ml)	Absorbance (450 nm)
5	3.486
2.5	2.215
1.25	1.172
0.625	0.726
0.313	0.454
0.156	0.330
0.078	0.232
0	0.156



### ASSAY PERFORMANCE

To assess the linearity of the assay, a serum sample containing naturally elevated NGAL at a concentration of 142 ng/ml was tested at dilutions ranging from 40- to 1280-fold to produce values within the dynamic range of the assay.



### REFERENCES

1. Steinbach S. et.al. Plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) in dogs with acute kidney injury or chronic kidney disease. *J. Vet. Intern. Med.* 28:264-269 (2014)

Rev 071619

For technical assistance please email us at  
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