



# LCN2 (Dog) ELISA Kit

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96 assay

Version: 02

Intended for research use only

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## **Introduction**

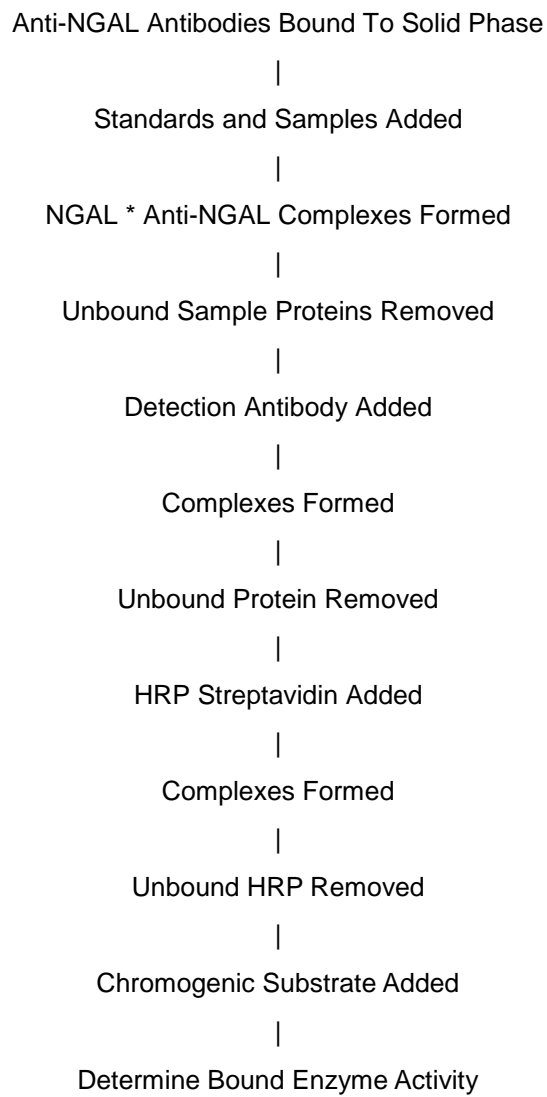
### **Intended Use**

The LCN2 (Dog) ELISA Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring NGAL in biological samples of dogs.

### **Principle of the Assay**

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the NGAL present in samples reacts with the anti-NGAL antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, the Detection Antibody, biotin conjugated anti-NGAL, is added and complexes are formed. Following a wash step, the horseradish peroxidase (HRP) conjugated Streptavidin is added and complexes are formed. After another washing step, the complexes are assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of NGAL in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of NGAL in the test sample. The quantity of NGAL in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Figure 1.



## General Information

### Materials Supplied

List of component

Component	Amount
Diluent Solution (Running Buffer): One bottle containing a 1X diluent running buffer.	60 mL
Wash Solution Concentrate: One bottle containing a 20X concentrated wash solution.	50 mL
Detection Antibody 100X: One vial containing affinity purified anti-Dog NGAL antibody conjugated with biotin in a stabilizing buffer.	150 µL
HRP-Streptavidin 100X: One vial containing HRP conjugated streptavidin in a stabilizing buffer	150 µL
Chromogen-Substrate Solution: One vial containing 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	12 mL
Stop Solution: One vial containing 0.3 M sulfuric acid. WARNING: Avoid contact with skin	12 mL
Anti-Dog NGAL ELISA Micro Plate: Twelve removable eight (8) well strips in well holder frame. Each well is coated with affinity purified anti-Dog NGAL.	96 (8x12) wells
Dog NGAL Calibrator: One vial containing a lyophilized Dog NGAL Calibrator.	1 vial

### Storage Instruction

The expiration date for the package is stated on the box label.

✓ Diluent Solution

The 1X Diluent Concentrate is stable until the expiration date and should be stored at 4-8°C.

✓ Wash Solution

The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

✓ Detection Antibody 100X

Undiluted Biotin conjugated anti-Dog NGAL should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.

✓ HRP-Streptavidin 100X

Undiluted horseradish peroxidase conjugated streptavidin should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.

✓ Chromogen-Substrate Solution

The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

✓ Stop Solution

The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

✓ Anti-Dog NGAL ELISA Micro Plate

Anti-Dog NGAL coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

✓ Dog NGAL Calibrator

The lyophilized Dog NGAL calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (Avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

**Materials Required but Not Supplied**

- ✓ Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- ✓ Test tubes
- ✓ Microplate washer/aspirator
- ✓ Distilled or Deionized H<sub>2</sub>O
- ✓ Microtitre plate reader
- ✓ Assorted glassware for the preparation of reagents and buffer solutions
- ✓ Timer

**Precautions for Use**

✓ Precaution

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

✓ Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

✓ Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

✓ Limitation of the procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
- Do not mix or substitute reagents with those from other lots or sources.

## Assay Protocol

### Reagent Preparation

✓ Diluent Solution

Ready to use as supplied.

✓ Wash Solution Concentrate.

The Wash Solution supplied is a 20X concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH<sub>2</sub>O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

✓ Detection Antibody 100X

Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL detection antibody to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

✓ HRP-Streptavidin 100X

Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL HRP-streptavidin to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

✓ Chromogen-Substrate Solution

Ready to use as supplied.

✓ Stop Solution

Ready to use as supplied.

✓ Anti-Dog NGAL ELISA Micro Plate

Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

✓ Dog NGAL Calibrator

Add 1.0 mL of distilled or deionized water to the Dog NGAL Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 20.0 ng/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Dog NGAL standards need to be prepared immediately prior to use (See chart below). Mix well between each step. Avoid foaming.

Standard	pg/mL	Volume added to 1X Diluent	Volume of 1X Diluent
7	1000	50 µL of NGAL Calibrator	950 µL
6	500	300 µL standard 7	300 µL
5	250	300 µL standard 6	300 µL
4	125	300 µL standard 5	300 µL
3	62.5	300 µL standard 4	300 µL
2	31.25	300 µL standard 3	300 µL

1	15.63	300 $\mu$ L standard 2	300 $\mu$ L
0	0		600 $\mu$ L

### **Sample Preparation**

#### ✓ Specimen Collection and Handling

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at  $-20^{\circ}\text{C}$ . Avoid repeated freeze thaw cycles.

#### ✓ Dilution of Samples

The assay for quantification of NGAL in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/100 is appropriate for most serum/plasma and urine samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/100 dilution of sample, transfer 5  $\mu$ L of sample to 495  $\mu$ L of 1X Diluent. This gives you a 1/100 dilution. Mix thoroughly. Dilute samples immediately prior to use.

### **Assay Procedure**

1. Bring all reagents to room temperature before use.
2. Pipette 100  $\mu$ L of
  - Standard 0 (0.0 ng/mL) in duplicate
  - Standard 1 (15.63 pg/mL) in duplicate
  - Standard 2 (31.25 pg/mL) in duplicate
  - Standard 3 (62.50 pg/mL) in duplicate
  - Standard 4 (125 pg/mL) in duplicate
  - Standard 5 (250 pg/mL) in duplicate
  - Standard 6 (500 pg/mL) in duplicate
  - Standard 7 (1000 pg/mL) in duplicate
3. Pipette 100  $\mu$ L of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for one hundred and twenty ( $120 \pm 2$ ) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then



pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100  $\mu$ L of appropriately diluted detection antibody to each well. Incubate the micro titer plate at room temperature for twenty ( $20 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100  $\mu$ L of appropriately diluted HRP-streptavidin to each well. Incubate the micro titer plate at room temperature for twenty ( $20 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.
10. Wash and blot the wells as described in Steps 5/6.
11. Pipette 100  $\mu$ L of TMB Substrate Solution into each well.
12. Incubate in the dark at room temperature for precisely ten (10) minutes.
13. After ten minutes, add 100  $\mu$ L of Stop Solution to each well.
14. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

✓ **Stability of the final reaction mixture**

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

## Data Analysis

### Calculation of Results

1. Subtract the average background value from the test values for each sample.
  2. Using the results observed for the standards construct a standard curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
  3. Interpolate test sample values from the standard curve. Correct for sera dilution factor to arrive at the NGAL concentration in original samples.
- ✓ Indication of instability
- If the test is performing correctly, the results observed with the standard solutions should be within 20% of the expected values.

**Resources**

**Plate Layout**

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