



**KAMIYA BIOMEDICAL COMPANY**

# Dog NGAL ELISA

**For the quantitative determination of NGAL (Lipocalin-2)  
in dog biological samples**

**Cat. No. KT-546**

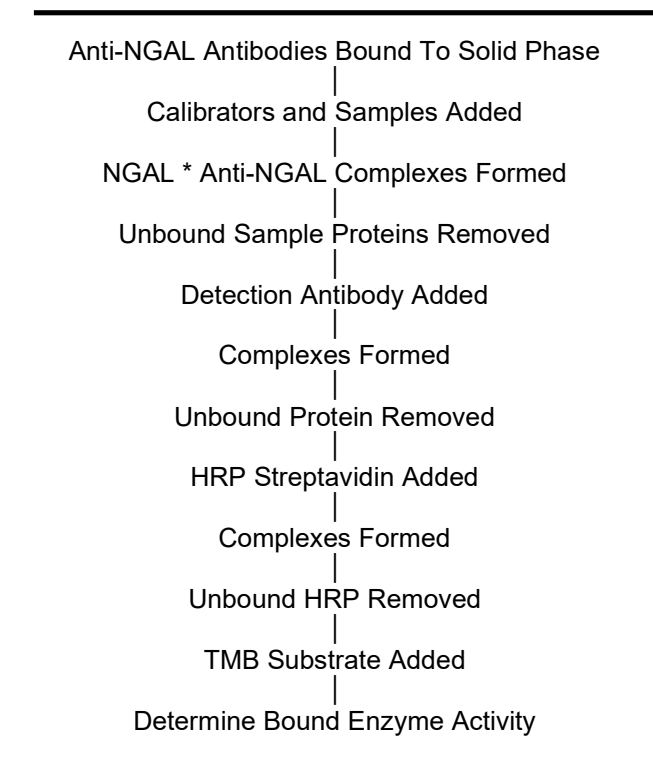
**For Research Use Only.**

**PRODUCT INFORMATION****Dog NGAL ELISA**  
**Cat. No. KT-546****INTENDED USE**

The Dog NGAL ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of NGAL (Lipocalin-2) in dog biological samples. For research use only.

**PRINCIPLE**

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the NGAL present in the samples reacts with the anti-NGAL antibody which has been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound sample 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 is proportional to 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 calibration curve constructed from the calibrators, and corrected for sample dilution.

**Figure 1.****COMPONENTS**

1. Diluent Solution  
One bottle containing 60 mL of a 1X diluent running buffer.
2. Wash Solution Concentrate  
One bottle containing 50 mL of a 20X concentrated wash solution.
3. Detection Antibody  
One vial containing 150 µL of a 100X concentrated affinity-purified anti-dog NGAL antibody conjugated with biotin in a stabilizing buffer.

4. HRP-Streptavidin  
One vial containing 150  $\mu$ L of a 100X concentrated HRP conjugated streptavidin in a stabilizing buffer.
5. TMB Substrate Solution  
One bottle containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.
6. Stop Solution  
One bottle containing 12 mL of 0.3 M sulfuric acid.  
**WARNING: Avoid contact with skin.**
7. Microtiter Plate  
Twelve removable eight-well strips in well holder frame. Wells are coated with affinity-purified anti-dog NGAL.
8. Dog NGAL Calibrator  
One vial containing a lyophilized Dog NGAL Calibrator.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2  $\mu$ L to 200  $\mu$ L) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microplate washer/aspirator
- Distilled or de-ionized H<sub>2</sub>O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Centrifuge for sample collection
- Anticoagulant for plasma collection

## PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
5. Other precautions:
  - Do not interchange kit components from different lots.
  - Do not use kit components beyond the expiration date.
  - Protect reagents from direct sunlight.
  - Do not pipette by mouth.
  - Do not eat, drink, smoke or apply cosmetics where reagents are used.
  - Avoid all contact with the reagents by using gloves.
  - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

## REAGENT PREPARATION

1. Diluent Solution  
Ready to use as supplied.
2. Wash Solution Concentrate  
The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. 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.
3. Detection Antibody 100X  
Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10  $\mu$ L detection antibody to 990  $\mu$ L of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. Dilute immediately before use and protect from light.
4. HRP-Streptavidin 100X  
Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10  $\mu$ L HRP-streptavidin to 990  $\mu$ L of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. Dilute immediately before use and protect from light.

4. TMB Substrate Solution  
Ready to use as supplied.
5. Stop Solution  
Ready to use as supplied.
6. Microtiter 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 with desiccant and re-seal.
7. Dog NGAL Calibrator  
Add 1.0 mL of distilled or de-ionized water to the Dog NGAL Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 8.0 ng/mL. The reconstituted calibrator should be aliquoted and stored frozen if future use is intended. Prepare the Dog NGAL Calibrators immediately prior to use according to the table below. Mix well between each step. Avoid foaming.

Calibrator	Concentration (pg/mL)	Calibrator Volume added to 1X Diluent	Volume of 1X Diluent
7	1,000	100 $\mu$ L Dog NGAL Calibrator	700 $\mu$ L
6	500	300 $\mu$ L Calibrator 7	300 $\mu$ L
5	250	300 $\mu$ L Calibrator 6	300 $\mu$ L
4	125	300 $\mu$ L Calibrator 5	300 $\mu$ L
3	62.50	300 $\mu$ L Calibrator 4	300 $\mu$ L
2	31.25	300 $\mu$ L Calibrator 3	300 $\mu$ L
1	15.63	300 $\mu$ L Calibrator 2	300 $\mu$ L
0	0		600 $\mu$ L

## STORAGE AND STABILITY

1. Complete Kit  
The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long term storage recommendations below for the Dog NGAL Calibrator.**
2. Diluent Solution  
The 1X Diluent Concentrate is stable until the expiration date and should be stored at 4°C.
3. 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 should be stored at 4°C.
4. Detection Antibody 100X  
Undiluted biotin conjugated anti-dog NGAL should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark. The undiluted conjugate is stable until the expiration date.
5. HRP-Streptavidin 100X  
Undiluted horseradish peroxidase conjugated streptavidin should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark. The undiluted conjugate is stable until the expiration date.
6. TMB Substrate Solution  
The TMB Substrate Solution should be stored at 4°C in the dark and is stable until the expiration date. Protect from light.
7. Stop Solution  
The Stop Solution should be stored at 4°C and is stable until the expiration date.
8. Microtiter Plate  
Anti-dog NGAL coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

### 9. 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 calibrator solutions should be prepared immediately prior to use.

## INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

## 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°C. Avoid repeated freeze-thaw cycles.

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

## ASSAY PROTOCOL

### 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/urine samples. For absolute quantification of samples that yield results outside the range of the calibration 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 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Mix thoroughly.

### Procedure

1. Bring all reagents to RT before use.

The Calibrators and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

2. Pipette 100 µL of
  - Calibrator 0 (0.0 pg/mL) in duplicate
  - Calibrator 1 (15.63 pg/mL) in duplicate
  - Calibrator 2 (31.25 pg/mL) in duplicate
  - Calibrator 3 (62.50 pg/mL) in duplicate
  - Calibrator 4 (125 pg/mL) in duplicate
  - Calibrator 5 (250 pg/mL) in duplicate
  - Calibrator 6 (500 pg/mL) in duplicate
  - Calibrator 7 (1,000 pg/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into pre-designated wells.
4. Incubate the Microtiter Plate at 22°C (RT) for one hundred and twenty (120 ± 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 diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.
7. Pipette 100 µL of appropriately diluted detection antibody to each well. Incubate at 22°C (RT) for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100  $\mu$ L of appropriately diluted HRP-streptavidin to each well. Incubate at 22°C (RT) 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 and 6.
11. Pipette 100  $\mu$ L of TMB Substrate Solution into each well.
12. Incubate in the dark at RT for precisely ten (10) minutes.
13. After ten minutes, add 100  $\mu$ L of Stop Solution to each well.
14. Determine the absorbance at 450 nm of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

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.

## RESULTS

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the calibrators construct a calibration 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 calibration curve. Correct for sample dilution factor to arrive at NGAL concentration in original sample.

## QUALITY CONTROL

In accord with good laboratory practice, the assays for specific NGAL require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

## LIMITATION OF THE PROCEDURE

1. 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.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

## **FOR RESEARCH USE ONLY**

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