# RAT NGAL

# CATALOG NUMBER: OKIA00157

Immunoperoxidase Assay for Determination of NGAL in Rat Samples

DIRECTIONS FOR USE

Version 4.0

***Please Read this Package Insert Completely Before Using This Product***

**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.

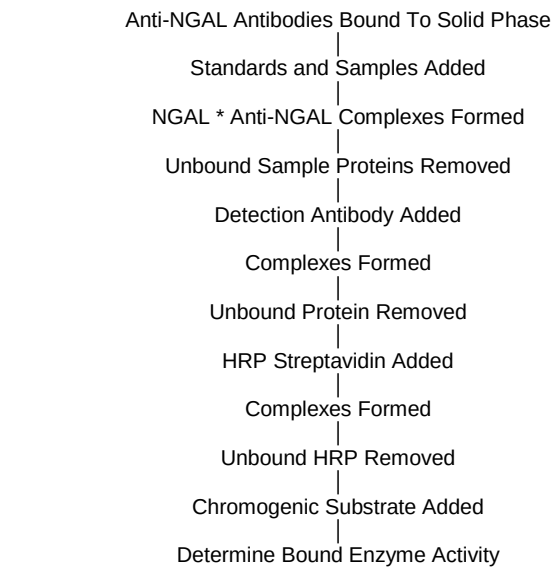



Figure 1

**INTENDED USE**

The NGAL test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring NGAL in Rat biological samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

## LIMITATION OF THE PROCEDURE

FOR RESEARCH USE ONLY. NOT FOR DISGNOSTIC PURPOSES. IN VITRO USE ONLY.
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 instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

## KIT COMPONENTS

**The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit protocol insert.**

| Component | Description | Preparation | Storage | Stability |
|---|---|---|---|---|
| **ELISA Micro Plate, antibody coated** | One plate of 12 removable 8 well strips, antibody coated | Ready to use as supplied. | 4-8°C, In sealed foil bag with desiccant | The plate strips are stable until the expiration date if stored correctly. |
| **Detection Antibody 100X** | One vial of 150uL of 100X affinity purified antibody conjugated with biotin in a stabilizing buffer | Dilute 1/100 immediately prior to use. | 4-8°C | The working solution should be diluted immediately prior to use and is stable up to one hour if stored in the dark. The 100X conjugate is stable until the expiration date. |
| **HRP-Streptavidin 100X** | One vial of 150uL of 100X Horseradish Peroxidase Conjugated streptavidin in a stabilizing buffer | Dilute 1/100 immediately prior to use. | 4-8°C | The working solution should be diluted immediately prior to use and is stable up to one hour if stored in the dark. The 100X conjugate is stable until the expiration date. |
| **Calibrator** | One vial of serum calibrator | Refer to the Certificate of Analysis (CoA). | 7 days or less: 4-8°C. More than 7 days: aliquot and freeze. Avoid multiple freeze-thaw cycles. | The working standard solutions should be prepared immediately prior to use. |
| **Diluent Solution** | One 50 mL bottle of 1X diluent buffer | Ready to use as supplied. | 4-8°C for both 1X working solution and X diluent concentrate | The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date. |
| **Wash Solution Concentrate** | One 50 mL bottle of 20X wash solution | Dilute 1/20 to make 1X working solution. | 4-8°C for both 1X working solution and 20X diluent concentrate | The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date. |
| **Chromogen-Substrate Solution** | One bottle of 12 ml 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3. | Ready to use as supplied | 4-8°C in the dark | Protect from light. The Substrate Solution is stable until the expiration date. |
| **STOP Solution** **WARNING: Avoid Contact with Skin** | One 12 ml bottle of 0.3 M sulfuric acid. | Ready to use as supplied | 4-8°C | The Stop Solution is stable until the expiration date. |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized $H_2O$
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

## SPECIMEN COLLECTION AND HANDLING

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

- Serum samples - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

- Plasma samples - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

- Urine samples – Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

## DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- Serum samples – Recommended starting dilution is 1/1,000. To prepare a 1/1,000 dilution of a sample, transfer 2 µL of sample to 198 µL of 1X diluent. This gives you a 1/100 dilution. Next dilute the 1/100 by transferring 30 µL into 270 µL of 1X diluent. This gives you a 1/1,000 dilution. Mix thoroughly at each stage.

- Plasma samples – Recommended starting dilution is 1/1,000. To prepare a 1/1,000 dilution of a sample, transfer 2 µL of sample to 198 µL of 1X diluent. This gives you a 1/100 dilution. Next dilute the 1/100 by transferring 30 µL into 270 µL of 1X diluent. This gives you a 1/1,000 dilution. Mix thoroughly at each stage.

- Urine samples – Recommended starting dilution is 1/1,000. To prepare a 1/1,000 dilution of a sample, transfer 2 µL of sample to 198 µL of 1X diluent. This gives you a 1/100 dilution. Next dilute the 1/100 by

transferring 30 µL into 270 µL of 1X diluent. This gives you a 1/1,000 dilution. Mix thoroughly at each stage.

**REAGENT PREPARATION**

- **Bring all reagents to room temperature (16-25°C) before use.**

- 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 dH2O). 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 - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Detection Antibody to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

- HRP-Streptavidin - 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. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

- Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil 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.

- Rat NGAL Calibrator – Prepare according to the Certificate of Analysis.

**ASSAY PROCEDURE**

1. **All samples and standards should be assayed in duplicates.**

2. Pipette 100 µL of
   Standard 0 (0.0 ng/ml) in duplicate
   Standard 1 (0.25 ng/ml) in duplicate
   Standard 2 (0.5 ng/ml) in duplicate
   Standard 3 (1 ng/ml) in duplicate
   Standard 4 (2 ng/ml) in duplicate
   Standard 5 (4 ng/ml) in duplicate
   Standard 6 (8 ng/ml) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for sixty (60 ± 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 µL of appropriately diluted Detection Antibody to each well. Incubate at room temperature 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 5/6.

9. Pipette 100 µL of appropriately diluted HRP-Streptavidin to each well. Incubate at room temperature for twenty (20 ± 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 µL 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 µL of Stop Solution to each well.

14. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

## CALCULATION OF RESULTS

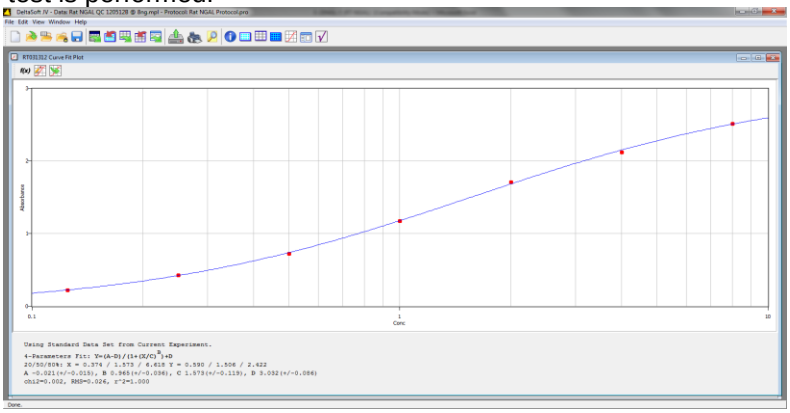
1. Subtract the average background value (Average absorbance reading of standard zero) from the test values for each sample.

2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the NGAL concentration in original samples.

## TYPICAL DATA

Sample Standard Curve is for demonstration only. Do not use for results calculations. A standard curve should be generated each time the test is performed.



| Standard # | Conc ng/mL | Replicates | Back Calc | % Recovery |
|---|---|---|---|---|
| 1 | 0.25 | 0.43 | 0.256 | 102.40 |
| 2 | 0.5 | 0.723 | 0.486 | 97.20 |
| 3 | 1 | 1.176 | 0.998 | 99.80 |
| 4 | 2 | 1.708 | 2.074 | 103.70 |
| 5 | 4 | 2.121 | 3.814 | 95.35 |
| 6 | 8 | 2.517 | 8.211 | 102.64 |

## PERFORMANCE CHARACTERISTICS

### 1. SENSITIVITY

The lowest detection limit (LDL) is 0.507 ng/mL.

The lowest detection limit is determined by adding three standard deviations to the mean optical density value of thirty-six zero standard replicates and calculating the corresponding concentration.

### 2. PRECISION

| | | |
|---|---|---|
| Intra-Assay Precision | Average %CV = 2.615 % | N = 36 |
| Inter-Assay Precision | Average %CV = 3.210 % | N = 36 |

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