Catalog #80687

V.1_91

Crystal Chem
High Performance Assays

# Rat NGAL ELISA Kit Instructions

For the quantitative determination of NGAL in
rat serum, plasma, and urine

**Catalog #80687**
**96 Assays**

**For research use only. Not for use in diagnostic procedures.**

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### *A. Intended Use*

The Rat NGAL ELISA kit is for the quantitative determination of NGAL in rat serum, plasma, and urine. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

### *B. Introduction*

Neutrophil gelatinase-associated lipocalin (NGAL) or liocalin-2 is a protein primarily expressed in the kidneys, prostate, and respiratory and GI tracts. Kidney injury can lead to high levels of NGAL being secreted within hours of the injury, making NGAL a useful biomarker for kidney health.

### *C. Principle of the Assay*

The Rat NGAL ELISA kit is a double antibody sandwich ELISA. An unknown amount of NGAL present in the sample binds with anti-NGAL antibodies adsorbed to the surface of the microplate. After washing to remove unbound proteins, anti-NGAL antibodies conjugated to biotin are added and form a complex with HRP conjugated to streptavidin and NGAL present in the wells. After another wash step, TMB substrate is added, and the concentration of NGAL is measured based on the resulting color intensity.

### *D. Kit Storage*

1. Upon receipt, store the kit at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

### *E. Assay Materials*

#### E.1. Materials provided

**TABLE 1 Contents of the kit**

| Mark | Description | Amount |
|---|---|---|
| MIC | Antibody-coated Microplate (12 x 8) | 1 pack |
| STD | Standard (Lyophilized) | 1 vial |
| DIL | Diluent (1X Concentrate) | 1 x 60 mL |
| BIOTIN | Antibody-Biotin conjugate (100X Concentrate) | 1 vial/150 µL |
| HRP | HRP-Streptavidin conjugate (100X Concentrate) | 1 vial/150 µL |
| WASH | Wash Buffer (20X Concentrate) | 1 x 50 mL |
| SUB | Substrate Solution | 1 x 12 mL |
| STOP | Stop Solution | 1 x 12 mL |

#### E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Volumetric flasks
Microplate reader (capable of reading $A_{450}$ and $A_{630}$ values)

### *F. Assay Precautions*

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

### *G. Maximizing Kit Performance*

1. Given the sample volumes required (2 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

### *H. Sample Collection*

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. Samples with excessive hemolysis should not be used. Samples containing azide or thimerosal are not compatible with this kit. Urine should be collected using standard methods and centrifuged to remove debris. The samples should be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

### *I. Assay Procedure*

#### I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standard
   The standard is provided in lyophilized form, and must be reconstituted with 1.0 mL of distilled or deionized water. The reconstituted standard concentration is 370.67 ng/mL. The standard should be stored frozen for future use and aliquoted in appropriate volumes prior to being frozen. Working standards should be prepared immediately prior to use as described in Section I.2. The working standard concentrations are 0, 0.25, 0.5, 1, 2, 4, and 8 ng/mL.
3. Diluent (1X Concentrated)
   Provided as ready to use.
4. Antibody-Biotin Conjugate (100X Concentrated)
   The Biotin Conjugate has to be diluted 1:100 with 1X Diluent prior to use. For each test strip, mix 10 µL of Biotin Conjugate with 990 µL of 1X Diluent. Mix uniformly, but gently. Avoid foaming. The Biotin Conjugate is stable for up to 1 hour when stored in the dark. Accordingly, working Biotin Conjugate should be prepared only as needed just prior to use.

5. HRP-Streptavidin Conjugate (100X Concentrated)
   The HRP Conjugate has to be diluted 1:100 with 1X Diluent prior to use. For each test strip, mix 10 µL of HRP Conjugate with 990 µL of 1X Diluent. Mix uniformly, but gently. Avoid foaming. The HRP Conjugate is stable for up to 1 hour when stored in the dark. Accordingly, working HRP Conjugate should be prepared only as needed just prior to use.
6. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized 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. Wash buffer is stable for at least one week after dilution.
7. Substrate Solution
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.

**I.2. Preparation of working standards**

1. Pipette 680 µL of 1X diluent and 15 µL of the standard (370.67 ng/mL) into a polypropylene microtube labeled 8 ng/mL, and mix thoroughly.
2. Pipette 300 µL of 1X diluent and 300 µL of the 8 ng/mL standard into a polypropylene microtube labeled 4 ng/mL, and mix thoroughly.
3. Dispense 300 µL of 1X diluent into four polypropylene microtubes labeled 2, 1, 0.5, and 0.25 ng/mL.
4. Dispense 300 µL of the 4 ng/mL standard into the 2 ng/mL microtube, and mix thoroughly.
5. Dispense 300 µL of the 2 ng/mL standard into the 1 ng/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 600 µL of 1X diluent into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 8, 4, 2, 1, 0.5, 0.25, and 0 ng/mL.

**Please note:** Working standards should be prepared immediately prior to use.

**I.3. Dilution of samples**

Samples need to be diluted with 1X diluent for use with the assay.

**Serum/Plasma/Urine Samples:** A sample dilution of 1:1,000 using 2 µL of sample is generally suitable. To prepare the 1:1,000 dilution, first mix 2 µL of sample with 198 µL of 1X Diluent to yield at 1:100 dilution. Next take 30 µL of the 1:100 dilution and mix with 270 µL of 1X Diluent to yield at 1:1000 dilution. Dilute sample immediately prior to use.

Since NGAL levels can vary, dilution ratios may need to be adjusted as appropriate.

**I.4. Assay procedure**

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or working standard.

2. Incubate plate for 60 mins at room temperature. Keep plate covered and level.
3. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. In each well, add 100 µL of diluted Biotin conjugate per well.
5. Incubate plate for 20 mins at room temperature. Keep plate covered in the dark and level.
6. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. In each well, add 100 µL of diluted HRP conjugate per well.
8. Incubate plate for 20 mins at room temperature. Keep plate covered in the dark and level.
9. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
10. Add 100 µL of the Substrate Solution in each well.
11. Incubate plate for 10 mins in a dark at room temperature.
12. Stop the reaction by adding 100 µL of Stop Solution.
13. Measure absorbance within 30 minutes using a plate reader (measure $A_{450}$ values and subtract $A_{630}$ values).

**I.5. Determining the NGAL concentration**

1. Using computer software, construct the NGAL calibration curve by plotting the background corrected mean absorbance value for each standard on the Y axis versus the corresponding NGAL concentration on the X axis. A four parametric logistic (4-PL) curve fit or second order polynomial (quadratic) are suitable for the evaluation. ***Note:*** *A calibration curve should be plotted every time the assay is performed.*
2. NGAL concentrations in the samples are interpolated using the calibration curve curve and background corrected mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor (*ie. 1,000*) to obtain the final NGAL concentration (expressed in ng/mL).
   ***Note:*** *Samples with high NGAL concentrations (ie. fall above the range of the assay) should be further diluted and rerun.*

***J. Performance characteristics***

**J.1. Assay range**

The Rat NGAL ELISA Kit has an assay range from 0.25 - 8 ng/mL.

**J.2. Precision**

The assay has a within-run and total precision of CV < 20%.

***Warranty***

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