

# **OmniKine™**

## **Human NGAL Colorimetric Sandwich ELISA Kit**

**Catalog #: OK-0339**

**Detection and Quantification of Human NGAL  
Concentrations in Supernatants, Sera and Plasma.**

**Research Purposes Only. Not Intended for Diagnostic or  
Clinical Procedures.**

**Store entire kit at 4°C until use.  
Kit expiration is 3 months from date of shipment**

**Manual Version: 1.8.622**

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## INTRODUCTION

Lipocalin-2/NGAL is an iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development. The protein binds iron through association with 2, 5-dihydroxybenzoic acid (2, 5-DHBA), a siderophore that shares structural similarities with bacterial enterobactin, and delivers or removes iron from the cell, depending on the context. The iron-bound form (holo-24p3) is internalized following binding to the SLC22A17 (24p3R) receptor, leading to release of iron and subsequent increase of intracellular iron concentration. In contrast, association of the iron-free form (apo-24p3) with the SLC22A17 (24p3R) receptor is followed by association with an intracellular siderophore, iron chelation and iron transfer to the extracellular medium, thereby reducing intracellular iron concentration. Lipocalin-2/NGAL is involved in apoptosis due to interleukin-3 (IL3) deprivation; the iron-loaded form increases intracellular iron concentration without promoting apoptosis, while the iron-free form decreases intracellular iron levels, inducing expression of the pro-apoptotic protein BCL2L11/BIM, resulting in apoptosis. This protein is also involved in innate immunity, possibly by sequestering iron, leading to limit bacterial growth. As a homodimer, it is disulfide-linked. As a heterodimer, it is disulfide-linked with MMP9. Lipocalin-2/NGAL is expressed in bone marrow and in tissues that are prone to exposure to microorganisms. High expression is found in bone marrow as well as in uterus, prostate, salivary gland, stomach, appendix, colon, trachea and lung. It is not found in the small intestine or peripheral blood leukocytes. Expression is activated by the oncoprotein BCR-ABL; BCR-ABL misregulates expression via the JAK/STAT pathway and binding of STAT5A to the promoter.

## ASSAY PRINCIPLES

The OmniKine™ Human NGAL ELISA Kit contains the components necessary for quantitative determination of natural or recombinant Human NGAL concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a “Sandwich” Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a “sandwich” format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator.

The capture antibodies coated to the bottom of each well are specific for a particular epitope on Human NGAL while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase.

After incubation and “sandwiching” of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'-Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration.

Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.

# ASSAY FORMAT

  
Capture Antibody



Capture antibodies specific for the target are coated to the plate. Additional binding sites on the plate are blocked.

  
Target Antigen



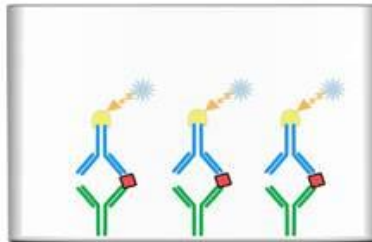
Target antigen present in standard or sample is bound by capture antibodies on the solid-phase.

  
Biotinylated Detection Antibody



Biotinylated detection antibodies specific for the target are added to bind another epitope on the target antigen.

  
Streptavidin-HRP



Streptavidin-HRP attaches to detection antibody via high affinity streptavidin-biotin interaction.

  
Unreacted TMB

  
Blue TMB Diimine Product



TMB substrate is converted to the blue TMB diimine via the HRP enzyme. Upon addition of acid, the reaction terminates and the wells can be read at 450 nm.

## MATERIALS INCLUDED

Component	Quantity Per Kit	Storage/Stability after first use
Microstrips Coated w/ Capture Antibody	12 x 8-Well Microstrips	1 month at 4°C
Protein Standard	Lyophilized	
Biotinylated Detection Antibody	Lyophilized	
400x Streptavidin-HRP B	30 µl	
Wash Buffer (15x)	50 ml	
Assay Diluent NM33	15 ml	
Assay Diluent LN28	50 ml	
Ready-to-Use Substrate	12 ml	
Stop Solution	12 ml	
Adhesive Plate Sealers	2 Sheets	
Technical Manual	1 Manual	-

**Any unused strips should be rewrapped with plate sealer and placed back into pouch with zipper closed until next use\*\***

## ADDITIONAL MATERIALS REQUIRED

The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm)
- Micropipettes ranging from 1 µl to 1 ml
- Distilled, deionized, and or purified water (recommended TOC 1-50 ppb, MΩ-cm 18.0)
- Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
- Graph paper or computer software capable of generating or displaying logarithmic functions
- Absorbent paper or vacuum aspirator
- Test tubes or microfuge tubes capable of storing ≥1 ml
- Bench-top centrifuge (optional)
- Bench-top vortex (optional)
- Orbital shaker (optional)

# SAMPLE PREPARATION AND STORAGE

Sample Type	Recommended Dilution Factor
Serum/ Plasma	1 to 20
Cell Culture Supernatant	1 to 5

Levels of Human NGAL may vary between samples. Optimal dilution factors for every sample must be determined by the investigator.

## ***Cell Supernatants***

Remove large cell components via centrifugation and perform the assay. Dilute cell supernatants at a minimum of 1 to 5 using Assay Diluent LN28 and assay.

## ***Serum***

Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. Dilute serum samples 1 to 20 using Assay Diluent NM33 and incubate 20 mins room temperature (RT) prior to continuing with the assay.

## ***Plasma***

Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. Dilute plasma samples 1 to 20 using Assay Diluent NM33 and incubate 20 mins prior to RT prior to continuing with the assay.

If samples are to be used within 24 hours, aliquot and store at 4°C. If samples are to be used over a long period of time, aliquot and store between -20°C and -80°C, depending on the duration of storage.

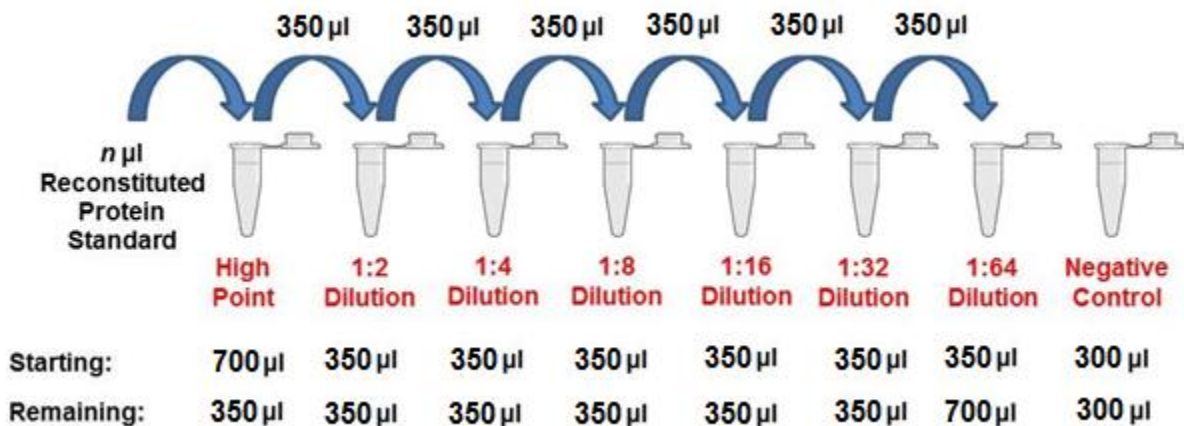
Samples containing a visible precipitate or pellet must be clarified prior to use in the assay.

Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples.

# REAGENT PREPARATION

All provided solutions should be at ambient temperature prior to use. **We recommend performing the assay in duplicate.** Reagents provided are enough to assay 96 wells and it is recommended to only prepare as much needed on the day of the experiment. All incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells.

1. Dilute the 15x Wash Buffer to 1x Wash Buffer using 14 volumes of ddH<sub>2</sub>O and 1 volume of 15x Wash Buffer.
2. Reconstitute Detection Antibody with 100 µl of ddH<sub>2</sub>O for a concentration of 100x. Mix gently and dilute to 1x prior to use with Assay Diluent LN28.
3. Reconstitute Protein Standard with 83 µl of ddH<sub>2</sub>O for a concentration of 192.77 ng/mL. Mix gently and dilute to the working range of the kit, 78.13-5000 pg/mL. To obtain a 7 point 2 fold dilution for the standard curve 18.15 µl of standard into 681.85 µl of diluent.
  - a. Dilute Protein Standard with Assay Diluent NM33 when experimenting with Serum/Plasma samples. **OR.**
  - b. Dilute Protein Standard with Assay Diluent LN28 when experimenting with Cell Supernatant samples.



4. Mix the 400x Streptavidin-HRP B (SAV-HRP) gently. Dilute to 1x using Assay Diluent LN28.
5. Stop Solution and Ready to Use Substrate are ready to use and do not need dilution.



## ASSAY PROCEDURE

1. Prepare all reagents to working concentrations, standards to desired range, and samples to appropriate dilution factors.
2. Remove desired number of capture antibody coated strips for experiment and place remaining strips back into dry pouch with desiccant for 4°C storage.
3. Add 100 µl of Standards/ Samples to each well and incubate on orbital shaker at room temperature (RT) for 2 hrs.
4. Aspirate the solution and add 300 µl of 1x Wash Buffer to each well being used and gently shake for 2-3 mins on an orbital shaker. Repeat this process 3 times. After the last wash ensure no liquid remains by inverting the plate and tapping it against clean paper towels.
5. Add 100 µl of 1x Detection Antibody to each well and incubate for 2 hrs. on an orbital shaker at RT.
6. Repeat step 4.
7. Add 100 µl of 1x SAV-HRP B to each well and incubate for 30 mins on an orbital shaker at RT
8. Repeat step 4.
9. Add 100 µl of Ready to use Substrate to each well, cover plate from light, and incubate for 20 mins on an orbital shaker at RT.
10. Add 100 µl of Stop Solution to each well and read at 450 nm.

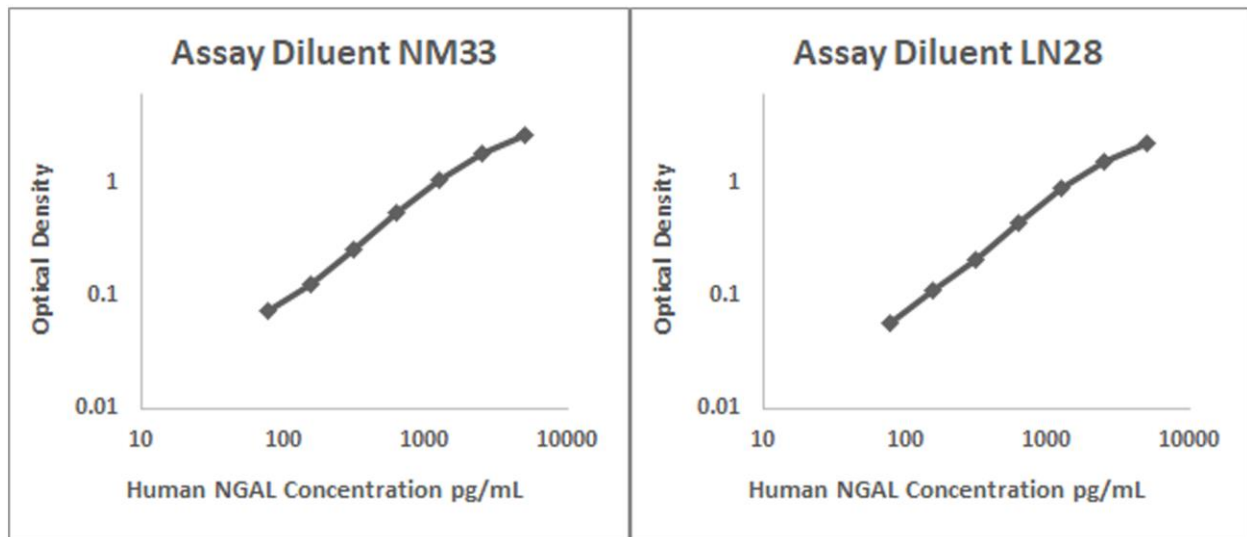
# DATA ANALYSIS

Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.

Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis).

Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or “trend-line” through the plotted points via regression analysis.

## TYPICAL DATA



<b>Concentration pg/ml</b>	5000.00	2500.00	1250.00	625.00	312.50	156.25	78.13
<b>Average Optical Density NM33</b>	2.66	1.80	1.05	0.54	0.25	0.13	0.07
<b>Average Optical Density LN28</b>	2.22	1.52	0.91	0.44	0.21	0.11	0.06

The data and subsequent graph were obtained after performing a cytokine ELISA for Human NGAL. Each known sample concentration was assayed in triplicate.

The standard curves shown are for demonstration. A new curve must be generated for each experiment.

## RECOVERY

Three concentrations of recombinant Human NGAL was spiked into various samples to measure the recovery percentage of recombinant protein in duplicate.

Sample Type	Average Recovery %	Range %
EDTA plasma (5%)	85	81-91
RPMI 10% FBS (20%)	103	98-106

## LINEARITY

Human NGAL recombinant protein was spiked into various biological samples and serial diluted with Assay Diluent NM33 for serum samples and Assay Diluent LN28 for RPMI 10% FBS.

		EDTA plasma	RPMI 10% FBS
Neat	pg/ml	568.49	660.93
	Expected %	722.00	106.00
1 to 2	pg/ml	384.22	333.96
	Expected %	722.00	107.00
1 to 4	pg/ml	183.68	156.53
	Expected %	117.00	100.00
1 to 8	pg/ml	153.78	68.22
	Expected %	94	87

## **SENSITIVITY**

The Human NGAL ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human NGAL proteins within the range of 78.13-5000 pg/ml.

## **CROSS REACTIVITY AND SPECIFICITY**

The OmniKine™ Human NGAL ELISA is capable of recognizing both recombinant and naturally produced Human NGAL proteins. The antigens listed below were tested at 50 ng/ml and did not exhibit significant cross reactivity or interference.

- Human: Lipocalin-1, MMP-9
- Murine: Lipocalin-2
- Rat: Lipocalin-2

# SUMMARIZED PROTOCOL

Reconstitute Biotinylated Detection Antibody and Protein Standard and dilute the 15x Wash Buffer as specified.



Perform serial dilution of Protein Standard and prepare samples as desired. See sample preparation section for instructions to dilute serum and plasma samples.



Add 100ul of Protein Standard, sample or control to each well and incubate for 2 hours at room temperature.



Aspirate Protein Standards, samples or controls out and wash plate 4 times.



Dilute Biotinylated Detection Antibody as specified. Add 100ul to each well and incubate for 2 hours at room temperature.



Aspirate Biotinylated Detection Antibody out and wash plate 4 times.



Dilute 400x Streptavidin-HRP as specified. Add 100ul of 1x Streptavidin-HRP to each well and incubate at room temperature for 30 minutes.



Aspirate 1x Streptavidin-HRP out and wash plate 4 times.



Add 100ul of Ready-to-Use Substrate to each well and incubate at room temperature for color development.



Add 100ul of Stop Solution and read plate at 450nm.

## HEALTH AND SAFETY PRECAUTIONS

- Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
- Stop Solution contains 2 N Sulfuric Acid ( $H_2SO_4$ ) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

## ASSAY RESTRICTIONS

- This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
- Individual results may vary due to differences in technique, plasticware and water sources.

# ELISA PLATE TEMPLATE

A	B	C	D	E	F	G	H	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

## TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.assaybiotech.com](http://www.assaybiotech.com) or contact us at:

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