EAGLE BIOSCIENCES

NGAL Plasma ELISA Kit

Catalog Number:
NGL31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 10 (31OCT24)

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INTENDED USE

The Eagle Biosciences NGAL Plasma ELISA Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of human neutrophil gelatinase-associated lipocaline (Lipocalin-2 or NGAL) in EDTA-plasma. The Eagle Biosciences NGAL Plasma ELISA Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

INTRODUCTION

NGAL or neutrophil gelatinase-associated lipocalin also know as Lipocalin-2 (LCN2) or oncogene 24p3 is a protein, which in humans is encoded by the LCN2 gene. NGAL is involved in innate immunity by sequestrating iron that in turn limits bacterial growth. It is expressed in neutrophils and in low levels in the kidney, prostate, and epithelia of the respiratory and alimentary tracts.

PRINCIPLE OF THE ASSAY

This Eagle Biosciences NGAL Plasma ELISA Kit is designed, developed and produced for the quantitative measurement of human NGAL in EDTA-plasma samples. The assay utilizes the "sandwich" technique with selected antibodies that bind to various epitopes of NGAL.

Assay standards, controls and subject samples are added directly to wells of a microtiter plate that is coated with antibody to human NGAL and incubated at room temperature for one hour. The plate is then washed and horseradish peroxidase (HRP) conjugated anti-NGAL is added to each well. After an additional incubation period, a "sandwich" of "solid-phase polyclonal antibody – human NGAL – HRP conjugated antibody" is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human NGAL in the test sample. A standard curve is generated by plotting the absorbance versus the respective human NGAL concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human NGAL in test samples is determined directly from this standard curve.

LIMITATIONS RELATED TO INTENDED USE

1. Subjects may have a higher than normal level of NGAL with:
    a. Systemic vasculitis
    b. Acute ischemic heart disease
    c. Other inflammatory diseases or infectious diseases
2. For sample values reading greater than the highest standard, it is recommended to re-assay samples with further dilutions (i.e. 1:10 or 1:100 with NGAL Sample Dilution Buffer).
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

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- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.5 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.

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- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS
The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS
Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT
Specimen Collection & Storage
EDTA-plasma samples are suitable specimens for human NGAL measurement. Only **10 µL** of human EDTA-plasma is required for a duplicate determination of human NGAL with this test kit. No special preparation of individual is necessary prior to specimen collection. EDTA-plasma should be collected by standard technologies of clinical laboratory practice and recommended by manufacturer of sample collection tube. It is extremely important to carefully separate the plasma from blood cells to avoid hemolyzation, etc. EDTA-plasma should be transferred to a clean test tube right after centrifugation. EDTA-plasma samples should be stored at 2-8°C if the assay is to be performed within 72 hours. Otherwise, subject samples should be stored at -20°C or below until measurement. Avoid more than three times freeze-thaw cycles of specimen. Do not use hemolyzed, hyperlipermic, heat-treated or any contaminated specimens.

Serum sample should not be used for NGAL measurement because the blood clotting process may lead to release NGAL from neutriphils, which could result in an unreliable test results.

Samples of heparin plasma and citrate plasma may be used for NGAL measurement.

Specimen Pre-Treatment
Specimen must be diluted 1:100 with diluted NGAL Sample Dilution buffer prior to use.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
- Precision single channel pipettes capable of delivering 100 µL
- Disposable pipette tips suitable for above volume dispensing.
- Aluminum foil.
- Distilled or deionized water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

REAGENTS PROVIDED

1. **Anti-NGAL Antibody Coated Microplate**

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Contents:	One 96-well microplate (12 x 8 strips) coated with polyclonal anti-human NGAL antibody.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

2. **Anti-NGAL Tracer Antibody (21x)**

Contents:	One bottle of horseradish peroxidase (HRP)-labeled anti-human NGAL antibody in a stabilized protein matrix.
Format:	Concentrated; Requires Preparation
Volume:	0.6 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.
Preparation:	**Dilute 1:21** with Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of the tracer antibody dilutent with 50 µL of the tracer antibody in a clean test tube. *Note: This **Antibody Working Solution** should be freshly prepared.*

3. **ELISA Wash Concentrate (30x)**

Contents:	One bottle containing surfactant in a phosphate-buffered saline with a non-azide, non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	30 mL
Storage:	2-25°C
Stability	Stable until the expiry date printed on the label.
Preparation:	**Dilute 1:30** with 870 mL of distilled or deionized water. Mix well before use.

4. **ELISA HRP Substrate**

Contents:	One bottle of tetramethylbenzidine (TMB) with stabilized hydrogen peroxide
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

5. **ELISA Stop Solution**

Contents:	One bottle containing an 0.5 M sulfuric acid.
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-25°C
Stability:	Stable until the expiry date printed on the label.

6. **Human NGAL Standards 1 – 6**

Contents:	Six bottles containing recombinant human NGAL in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to the vials for exact concentration of the standard.**
Format:	Lyophilized; Requires Preparation
Storage:	2-8°C (Lyophilized), -20°C (Reconstituted)

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Stability	Stable until the expiry date printed on the label. After reconstitution: stored at 2-8°C for three days, below -20°C for long term storage. Do not exceed 3 freeze-thaw cycles.
Preparation:	Reconstitute assay standards by adding **1.0 mL** of demineralized water to each standard bottle. Allow the standard to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use.

7. **Human NGAL Controls 1 – 2**

Contents:	Two bottles containing recombinant human NGAL in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to the vials for exact concentration for each control.**
Format:	Lyophilized; Requires Preparation
Storage:	2-8°C (Lyophilized), -20°C (Reconstituted)
Stability	Stable until the expiry date printed on the label. After reconstitution: stored at 2-8°C for three days, below -20°C for long term storage. Do not exceed 3 freeze-thaw cycles.
Preparation:	Reconstitute assay controls by adding **1.0 mL** of demineralized water to each control bottle. Allow the control to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use.

8. **Tracer Antibody Diluent**

Contents:	One bottle containing ready to use buffer to be used only for tracer antibody dilution according to the assay procedure.
Format:	Ready to Use
Volume:	12 mL
Storage:	2-8°C
Stability	Stable until the expiry date printed on the label.

9. **Concentrated NGAL Sample Extraction Buffer (2x)**

Contents:	One bottle containing concentrated buffer matrix with protein stabilizers and preservative.
Format:	Concentrated; Requires Preparation
Volume:	30 mL/ bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.
Preparation:	**Dilute 1:2** with 30 mL of distilled or deionized water. Mix well before use.

RECOMMENDED ASSAY LAYOUT*

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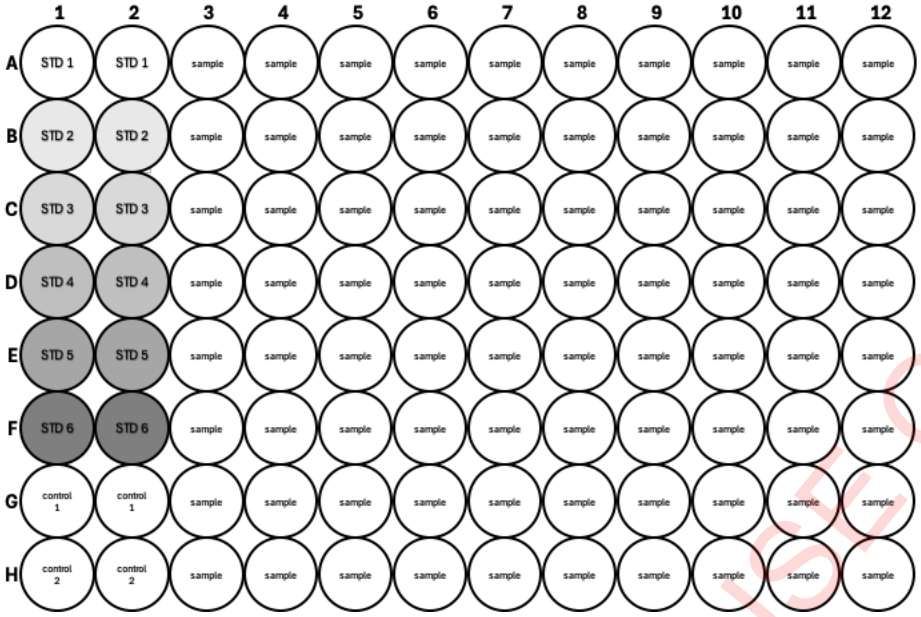

***** *Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Place a sufficient number of microwell strips in a holder to run standards, controls, and samples in duplicate.
2. **Add 100 µL** of standards, controls and diluted subject samples into the designated microwells.
3. Cover the plate with one plate sealer and aluminum foil. Incubate the plate at **room temperature (20-25°C)** for **60 minutes.**
4. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
5. Prepare the Antibody Working Solution as stated in *Reagents Provided* section.
6. **Add 100 µL** of the Antibody Working Solution to each well. Mix by gently tapping the plate.
7. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25°C)** for **30 minutes.**
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing **350 µL** of diluted wash solution into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. **Add 100 µL** of ELISA HRP Substrate into each of the wells. Mix by gently tapping the plate.
10. Cover the plate with one plate sealer and aluminum foil. Incubate the plate at **room temperature (20-25°C)** for **20 minutes.**
11. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix by gently tapping the plate.
12. Read the absorbance at 450/620 nm or 450/650 nm within 10 minutes.

INTERPRETATION OF RESULTS

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

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1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
4. The human NGAL concentrations for the controls are read directly from the standard curve using their respected corrected absorbance.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

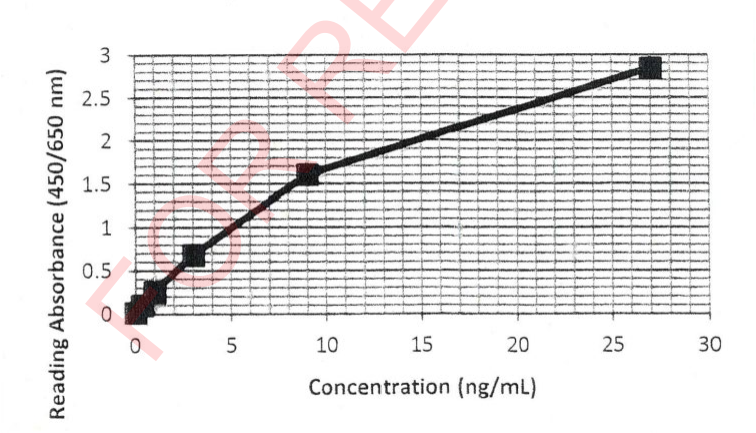
EXPECTED VALUES

EDTA-plasma samples from normal healthy adults with age 20-60 were collected and measured with this ELISA. The recommended normal high cut-off for NGAL concentration by using this ELISA is 5 ng/mL with an average level >1.06 ng/mL (range 0.48-3.9 ng/mL, SD>0.56 ng/mL). We strongly recommend for each laboratory to establish its own normal range by measuring EDTA-plasma with this ELISA Kit.

TYPICAL DATA

A typical absorbance data and the resulting standard curve from this NGAL Plasma ELISA Kit are represented. This curve should not be used in lieu of standard curve generated with each assay.

Well I.D.	Reading Absorbance (450 nm)		Concentration (ng/mL)
	Average	Corrected	
STD 1: 0 ng/mL	0.017	0.000	-
STD 2: 0.3 ng/mL	0.104	0.087	-
STD 3: 1 ng/mL	0.254	0.237	-
STD 4: 3 ng/mL	0.687	0.687	-
STD 5: 9 ng/mL	1.605	1.605	-
STD 6: 27 ng/mL	2.846	2.846	-
Control 1	0.468	0.468	1.991
Control 2	1.251	1.251	6.688



PERFORMANCE AND CHARACTERISTICS

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Sensitivity
The analytical sensitivity (LLOD) of the NGAL ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.04 ng/mL.

High Dose "hook" effect
This assay has showed that it did not have any high dose "hook" for NGAL level up to 18,000 ng/mL.

Intra-Assay Precision
The intra-assay precision was validated by measuring three diluted 1:100 control samples with 16 replicate determinations. The results are as follows:

Sample #	Mean (ng/mL)	CV (%)
1	0.648	5.1
2	1.735	7.2
3	5.262	7.9

Inter-Assay Precision
The inter-assay precision was validated by measuring two control levels in duplicate in 14 individual assays. The results are as follows:

Sample #	Mean (ng/mL)	CV (%)
1	6.507	5.1
2	2.098	6.9

Linearity
Two human serum samples from dialysis patients were diluted with a BSA based 0.01M phosphate, 0.15M sodium chloride buffer matrix and assayed. The results are as follows:

Samples	Observed (ng/mL)	Recovery (%)
Sample A	12.1	-
50%	6.1	101
25%	3.1	102
12.5%	1.6	104
Sample B	6.2	-
50%	2.9	94
25%	1.5	98
12.5%	0.8	102
Sample C	24.7	-
50%	15.3	124
25%	17.3	118
12.5%	3.2	105

REFERENCES

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5. Friedl A, Stoesz SP, Buckley P, Gould MN (July 1999). "Neutrophil gelatinase-associated lipocalin in normal and neoplastic human tissues. Cell type-specific pattern of expression". Histochem. J. 31 (7): 433–41.

WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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