EAGLE BIOSCIENCES

NGAL (Plasma) ELISA Assay Kit

Catalog Number:
NGL31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 11.0

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INTENDED USE

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

Indications for use:
Patient may have a higher than normal level of NGAL with
1. Systemic vasculitis
2. Acute ischemic heart disease
3. Other inflammatory diseases or infectious diseases

This kit is for research use only.

SUMMARY OF PHYSIOLOGY

NGAL or neutrophil gelatinase-associated lipocalin also known as Lipocalin-2 (LCN2) or oncogene 24p3 is a protein, which in humans is encoded by the LCN2gene. 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.

ASSAY PRINCIPLE

This Human NGAL ELISA Assay 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 calibrators, controls and patient 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.

REAGENTS: Preparation and Storage

The NGAL ELISA Assay Kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

1. Anti-NGAL Antibody Coated Microplate

Microplate coated with polyclonal anti-human NGAL antibody.
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. HRP Conjugated Anti-NGAL Antibody

HRP-labeled anti-human NGAL antibody in a stabilized protein matrix
Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: 21X Concentrate. This reagent must be diluted with tracer antibody diluent and mixed well before use..

3. ELISA Wash Concentrate

Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use..

4. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

5. ELISA Stop Solution

0.5 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use.

6. Human NGAL Calibrators Levels 1 to 6

Recombinant human NGAL in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to each vial for exact concentration.
Qty: 6 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C(Reconstituted) Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 1.0 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

7. Human NGAL Controls

Recombinant human NGAL in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to each vial for exact concentration.
Qty: 2 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C(Reconstituted) Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 1.0 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

8. Tracer Antibody Diluent

For tracer antibody dilution.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

9. NGAL Sample Dilution Buffer

Concentrated buffer matrix with protein stabilizers and preservative.
Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 2X concentrate. Before use the concentrated buffer must be diluted with 30 mL of demineralized water and mixed well.

SAFETY PRECAUTIONS

The NGAL ELISA Assay Kit reagents are for in research use only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 100 µL.
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multichannel wash bottle or automatic (semi-automatic) washing system
7. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm

SPECIMEN COLLECTION

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 – 8ºC if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at –20ºC 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.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

(3) Reconstitute assay calibrators and controls by adding 1.0 mL of deminerialized water to each calibrator and control bottle. Allow the calibrator and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted calibrators and controls may be stored at 2- 8°C for up to 3 days or below –20°C for long-term storage. Do not exceed 3 freeze-thaw cycles.

(4) Concentrated Patient Sample Diluent must be diluted to working solution prior to use. Please see REAGENTS section for details.

2. Specimen Preparation

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

3. Assay Procedure

(1) Place a sufficient number of microwell strips in a holder to run calibrators and controls, and samples in duplicate.

(2) Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
C	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
D	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
E	Calibrator Level 3	Control 1	SAMPLE 3
F	Calibrator Level 3	Control 1	SAMPLE 3
G	Calibrator Level 4	Control 2	SAMPLE 4
H	Calibrator Level 4	Control 2	SAMPLE 4

(3) Add **100 µL** of calibrators and controls, and diluted samples into the designated microwells.

(4) Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC) for 60 minute**s.

(5) 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.

(6) Prepare Antibody working solution by 1:21 fold dilution of the tracer antibody with the diluent. For each strip, it is required to mix 1 mL of the tracer antibody diluent with 50 µL of the tracer antibody in a clean test tube.
Note: This Antibody working solution should be freshly prepared.

(7) Add **100 µL** of the *Antibody working solution* into each of the wells. Mix by gently tapping the plate.

(8) Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**

(9) 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.

(10) Add **100 µL** of ELISA HRP Substrate into each of the wells. Mix by gently tapping the plate.

(11) Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**

(12) 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.

(13) Read the absorbance at **450/620 nm** or **450/650 nm** within **10 minutes** with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
8. If adapting this assay to automated ELISA system such as DS-2 (Diamedix Corp., Miami), a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION OF RESULTS

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

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 calibrator levels on the ordinate against the calibrator 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 respective corrected absorbance.

LIMITATION OF THE PROCEDURE

1. An abnormally high NGAL test result cannot be independently used for clinical diagnosis. As with other laboratory tests, a variety of analytical and pre-analytical factors may lead to false high test results. Physicians must interpret the test result in the light of each patient's clinical findings.
2. For sample values reading greater than the highest calibrator, 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.

QUALITY CONTROL

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

EXPECTED VALUES

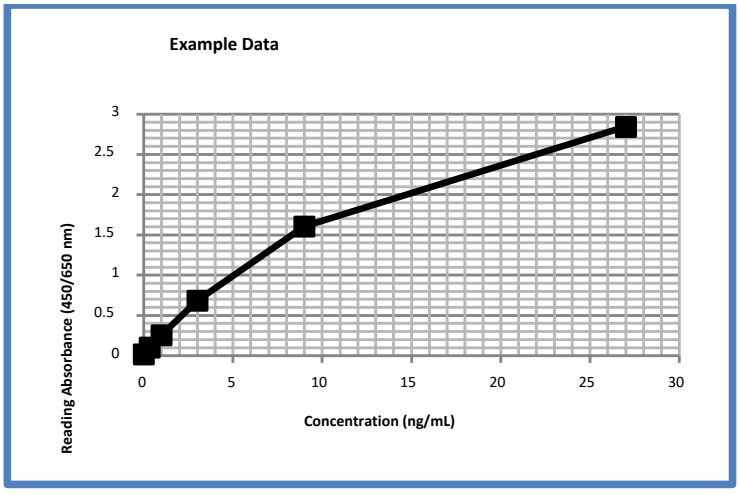
EDTA plasma samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The recommended normal high cut-offfor NGAL concentration by using this ELISA is 500 ng/mL with an average level >106 ng/mL (range 48 – 390 ng/mL, SD>56 ng/mL). We strongly recommend for each clinical laboratory to establish its own normal range by measuring EDTA plasma with this ELISA.

EXPECTED VALUES

A typical absorbance data and the resulting standard curve from this NGAL ELISA are represented.

Note: This curve should not be used in lieu of calibrator curve run with each assay.

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



QUALITY CONTROL

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

PERFORMANCE CHARACTERISTICS

Sensitivity

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

Hook Effect

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

Reproducibility and Precision

The intra-assay precision was validated by measuring three diluted 1:100 control samples with 16 replicate determinations The inter-assay precision was validated by measuring two control levels in duplicate in 14 individual assays. The results are as follows: .

	Intra-Assay			Inter-Assay	
Sample	**1**	**2**	**3**	**1**	**2**
Mean (ng/mL)	0.648	1.735	5.262	6.507	2.098
CV (%)	5.1	7.2	7.9	5.1	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

1. Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N (May 1993). "Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase". J. Biol. Chem. 268 (14): 10425–32.
2. Chan P, Simon-Chazottes D, Mattei MG, Guenet JL, Salier JP (September 1994). "Comparative mapping of lipocalin genes in human and mouse: the four genes for complement C8 gamma chain, prostaglandin-D-synthase, oncogene-24p3, and progestagen-associated endometrial protein map to HSA9 and MMU2". Genomics 23(1): 145–50.
3. Cowland JB, Borregaard N (October 1997). "Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans". Genomics 45 (1): 17–23.
4. Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, Du T, Erdjument-Bromage H, Tempst P, Strong R, Barasch J
   (November 2002). "An iron delivery pathway mediated by a lipocalin". Mol. Cell 10 (5): 1045–56.
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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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

SHORT ASSAY PROCEDURE

1. Add **100 µL** of the calibrators, controls, and diluted samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the antibody working solution into the designated microwells.
5. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
6. Add **100 µL** of substrate to each well.
7. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
8. Add **100 µL** of the stop solution to each well.
9. Read the absorbance at **450/620 nm** or **450/650 nm.**