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EDI™ Human NGAL ELISA

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Human Neutrophil Gelatinase-Associated Lipocalin Levels in EDTA-Plasma

REF **KT-883** EU: IVD CE Σ 96 +2 +8 °C

INTENDED USE
This test kit is intended for use in the quantitative determination of human neutrophil gelatinase-associated lipocalin (Lipocalin-2 or NGAL) in EDTA-plasma.

Indications for use:

Patient may have a higher than normal level of NGAL with

1. Acute kidney failure
2. Systemic vasculitis
3. Acute ischemic heart disease
4. Other inflammatory diseases or infectious diseases

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 *LCN2* gene. NGAL is involved in innate immunity by sequestrating iron that in turn limits bacterial growth.[4] It is expressed in neutrophils and in low levels in the kidney, prostate, and epithelia of the respiratory and alimentary tracts. Studies have shown that NGAL is an early biomarker for ischaemic renal injury after cardiopulmonary bypass.

ASSAY PRINCIPLE
This 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 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
This test 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.

Prior to use, allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. Anti-NGAL Antibody Coated Microplate (Cat. No. 30641)
One microplate with twelve by eight strips (96 wells total) coated with polyclonal anti-human NGAL antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. HRP Conjugated Anti-NGAL Antibody (Cat. No. 30650)
One vial containing **0.6 mL** HRP-labeled anti-human NGAL antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. ELISA Wash Concentrate (Cat. No. 10010)
One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

4. ELISA HRP Substrate (Cat. No. 10020)
One bottle containing 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. ELISA Stop Solution (Cat. No. 10030)
One bottle containing 12 mL of stop solution. This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

6. Human NGAL Standards (Cat. No. 30642 – 30647)
Six vials 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.** These standards should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for dilution direction.

7. Human NGAL Controls (Cat. No. 30648 – 30649)
Two vials containing human NGAL in a lyophilized bovine serum based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.

8. Tracer Antibody Diluent (Cat. No. 30651)
One bottle containing **12 mL** ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

9. NGAL Sample Dilution Buffer (Cat. No. 30654)
One bottle contains **30 mL** of 2-fold concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted with **30 mL** of demineralized water and mixed well.

SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum 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 are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

(3) Reconstitute assay standards and controls by adding **1.0 mL** of demineralized water to each standard and control bottle. Allow the standard 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 standards 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 (Cat. 30654) must be diluted to working solution prior to use. Please see REAGENTS section for details.

(5) Each unknown sample needs to be diluted **1:100** using **1x NGAL Sample Dilution Buffer** as a sample diluent.

(6) Prepare Tracer Antibody working solution by **1:21** fold dilution of the NGAL Tracer Antibody (Cat. 30650) by adding the tracer antibody into the Tracer Antibody Diluent (Cat. 30651). Following is a table that outlines the relationship of strips used and antibody mixture prepared. ***NOTE:*** *the tracer antibody should be prepared just prior to the end of the first incubation cycle.*

Dilution Scheme	Tracer Antibody Diluent	Tracer Antibody
1	1 mL	50 µL
2	2 mL	100 µL
3	3 mL	150 µL
4	4 mL	200 µL
5	5 mL	250 µL
6	6 mL	300 µL
7	7 mL	350 µL
8	8 mL	400 µL
9	9 mL	450 µL
10	10 mL	500 µL
11	11 mL	550 µL
12	12 mL	600 µL

(7) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	SAMPLE 1	SAMPLE 5
B	STD 1	STD 5	SAMPLE 1	SAMPLE 5
C	STD 2	STD 6	SAMPLE 2	SAMPLE 6
D	STD 2	STD 6	SAMPLE 2	SAMPLE 6
E	STD 3	C 1	SAMPLE 3	
F	STD 3	C 1	SAMPLE 3	
G	STD 4	C 2	SAMPLE 4	
H	STD 4	C 2	SAMPLE 4	

(8) Place a sufficient number of Anti-NGAL antibody-coated microwell strips (Cat. 30641) in a holder to run human

NGAL standards, controls and unknown samples in duplicates.

2. Assay Procedure:

(1) Add **100 µL** of Standards, Controls and diluted patient samples (diluted beforehand 1:100 with NGAL Sample Dilution Buffer, Cat. 30654) into the designated microwells.
(2) Seal the plate wells securely, cover with foil or other material to protect from light. Incubate the plate static, at room temperature for **1 hr. ± 5 minutes**.
(3) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(4) Dilute the proper amount of Tracer Antibody for the assay.
(5) Add **100 µL** of the above Tracer Antibody to each well.
(6) Seal the plate wells securely, cover with foil or other material to protect from light. Incubate the plate static, at room temperature for **30 minutes ± 5 minutes**.
(7) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(8) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(9) Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate the plate static, at room temperature for **20 minutes**.
(10) Immediately add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(11) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, 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.

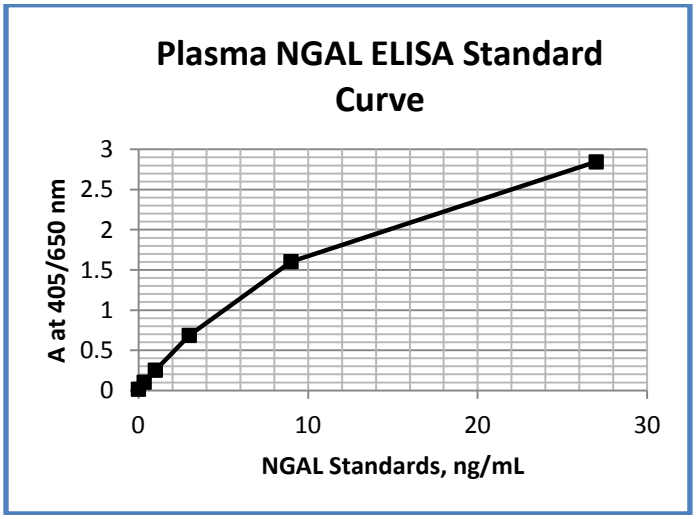
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard 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.

The human NGAL concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

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

Well I.D.	OD 450/650 nm Absorbance			Results
	Readings	Average	Corrected	
Std-1: 0 ng/mL	0.017 0.017	0.017	0.000	
Std-2: 0.3 ng/mL	0.106 0.102	0.104	0.087	
Std-3: 1 ng/mL	0.256 0.252	0.254	0.237	
Std-4: 3 ng/mL	0.679 0.694	0.687	0.670	
Std-5: 9 ng/mL	1.597 1.614	1.605	1.588	
Std-6: 27 ng/mL	2.810 2.881	2.846	2.829	
Control 1	0.491 0.446	0.468	0.451	1.991 ng/mL
Control 2	1.245 1.257	1.251	1.234	6.688 ng/mL



EXPECTED VALUES

EDTA plasma samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The recommended normal high cut-off for 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.

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 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.

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 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 levels up to 18,000 ng/mL.

Precision

The intra-assay precision was validated by measuring three diluted 1:100 control samples with 16 replicate determinations.

Sample 1:100	Mean NGAL Value (ng/mL)	CV (%)
1	0.648	5.1
2	1.735	7.2
3	5.262	7.9

The inter-assay precision was validated by measuring two control levels in duplicate in 14 individual assays.

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

Linearity

Three **EDTA plasma** samples were collected, diluted 1:100 and spiked with various amounts of NGAL, diluted with standard zero matrix and tested. The results of NGAL percent recovery value in ng/mL are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
1:100 Sample A	12.1	-
1:2	6.1	101
1:4	3.1	102
1:8	1.6	104
1:100 Sample B	6.2	-
1:2	2.9	94
1:4	1.5	98
1:8	0.8	102
1:100 Sample C	24.7	-
1:2	15.3	124
1:4	17.3	118
1:8	3.2	105

Spike Recovery

Two diluted 1:100 EDTA plasma samples and three assay standards (1, 3 and 9 pg/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
1:100 Sample A	0.5	-
Std-3: 1 ng/mL	0.8	106
Std-4: 3 ng/mL	1.9	108
Std-5: 9 ng/mL	5.2	109
1:100 Sample B	1.0	-
Std-3: 1 ng/mL	1.1	112
Std-4: 3 ng/mL	2.1	103
Std-5: 9 ng/mL	5.4	108

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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This product is developed and manufactured by
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Schiffgraben 41
30175 Hannover, Germany

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

NGAL ELISA: Condensed Assay Protocol

1. 100 µL Standards, controls, and 1:100 diluted patient samples

Incubate @ RT for 60 min
static
Wash 5 x

2. 100 µL Tracer Antibody

Incubate @ RT for 30 min
static
Wash 5 x

3. 100 µL TMB Substrate

Incubate @ RT for 20 min
static

Immediately

4. 100 µL Stop Solution

5. Read absorbance at 450/650 or 450/620 nm

within 10 minutes

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