（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !）

QuicKey Human NGAL(Neutrophil Gelatinase Associated Lipocalin) ELISA Kit

Catalog No: E-TSEL-H0003

96T/48T/24T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Phone: 240-252-7368(USA) 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please refer to specific expiry date from label on the side of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

QuicKey Series

Get more sensitive and precise results with saving at least 1h comparing to traditional ELISA Kits. The new developed technology in house will help to accelerate your science research in a more efficient way.

Intended use

This ELISA kit applies to the in vitro quantitative determination of Human NGAL concentrations in serum, plasma, urine. Please consult technical support for the applicability if other biological fluids need to be tested.

Specification

- Sensitivity: 0.06ng/mL.
- Detection Range: 0.13-8ng/mL
- Specificity: This kit recognizes Human NGAL in samples. No significant cross-reactivity or interference between Human NGAL and analogues was observed.
- Repeatability: Coefficient of variation is < 10%.

Background

Neutrophil gelatinase associated lipocalin (NGAL) also known as Lipocalin 2 or Lcn2 is a 25 kDa, is a member of the lipocalin superfamily and a pleiotropic mediator of various inflammatory processes [1].NGAL, identified originally as a protein associated with matrix metalloproteinase 9 (MMP-9) of human neutrophils. Lipocalins are extracellular proteins which share a common tertiary structure that forms a barrel-like hydrophobic ligand binding site. When bound to MMP-9, NGAL protects it from proteolytic degradation sustaining the proteolytic activity of MMP-9. Neutrophil gelatinase-associated lipocalin (NGAL), is released by various cell types and is an attractive biomarker of inflammation, ischemia, infection, and kidney damage. Both intestinal and metabolic inflammation, as observed in obesity and related disorders, are associated with increased NGAL synthesis [2]. While NGAL in the intestinal tract regulates the composition of the gut microbiota and shows anti-inflammatory activities, it also exhibits proinflammatory activities in other experimental settings. Plasma NGAL is a novel marker of kidney function, which correlates to duration of end-stage renal failure (ESRD) and serum creatinine in uremic patients awaiting kidney transplantation. Plasma NGAL is associated with homocysteine in transplanted patients.

1. Kjeldsen L, Cowland J B, Borregaard N. Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse.[J]. Biochim Biophys Acta, 2000, 1482(1):272-283.

2. Tilg, H., & Moschen, A. R. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nature Reviews Immunology, 2006, 6(10), 772.

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human NGAL. Samples (or Standards) and biotinylated detection antibody specific for Human NGAL are added to the micro ELISA plate wells. Human NGAL would combine with the specific antibody. Then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human NGAL, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ±2 nm. The OD value is proportional to the concentration of Human NGAL. You can calculate the concentration of Human NGAL in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for six months. After test, the unused wells and reagents should be stored according to the table below.

Item	Specifications	Storage conditions after test
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	2-8℃, 1 month
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	Discard unused reconstituted standard and dilutions
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8℃
Biotinylated Detection Ab Working Solution	1 vial, 6 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 μL 48T: 1 vial, 60 μL 24T: 1 vial, 60 μL	2-8℃(Protect from light)
Substrate Reagent	1 vial, 10 mL	
Stop Solution	1 vial, 10 mL	2-8℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch provided in the kit, store it according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard and HRP conjugate working solution. The unspent biotinylated detection Ab working solution and other stock solutions should be stored according to the storage conditions suggested in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±10 nm. The optical density should be within 0~3.5.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

(More detailed information please view our website: http://www.elabscience.com/List-detail-253.html)

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

Urine: Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2~8 ℃.Collect the supernatant to carry out the assay.

Note for sample

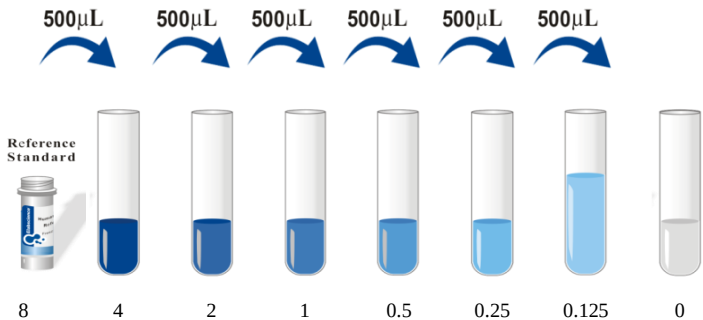
1. Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

3. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 8ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 8、4、2、1、0.5、0.25、0.125、0ng/mL.

   Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 8ng/mL working solution to the first tube and mix up to produce a 4ng/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 12th page)

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 μL for each well). Add the samples to the other wells (50 μL for each well). Immediately add 50μL of **Biotinylated Detection Ab working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃.Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Aspirate or decant the solution from each well, add 350μL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
3. Add 100μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37℃.
4. Aspirate or decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
5. Add 90μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
6. Add 50μLof **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm

Calculation of results

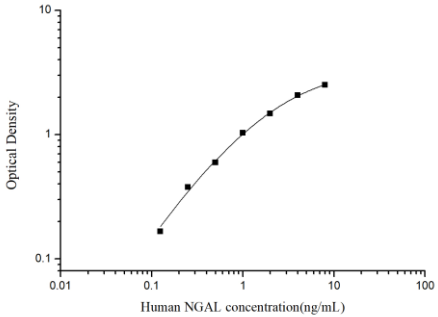
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(ng/mL)	8	4	2	1	0.5	0.25	0.125	0
OD	2.569	2.130	1.533	1.088	0.656	0.437	0.225	0.059
Corrected OD	2.510	2.071	1.474	1.029	0.597	0.378	0.166	-



Sample values

Serum/Plasma/Urine–Samples from apparently healthy volunteers were evaluated for the presence of Human NGAL in this assay.

Sample Type	Source	Range	Dilution Factor
Serum(n=19)	Healthy human	68.47-210.56ng/mL	50-500
EDTA plasma (n=12)	Healthy human	50-180.8ng/mL	50-500
Urine(n=17)	Healthy human	0.52-75ng/mL	1-50

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human NGAL were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human NGAL were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean((ng/mL)	0.37	0.90	3.57	0.35	0.81	3.73
Standard deviation	0.02	0.04	0.16	0.02	0.04	0.17
CV (%)	5.41	4.44	4.48	5.71	4.94	4.56

Recovery

The recovery of Human NGAL spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	86-100	94
EDTA plasma (n=8)	101-110	104
Urine(n=8)	87-98	90

Linearity

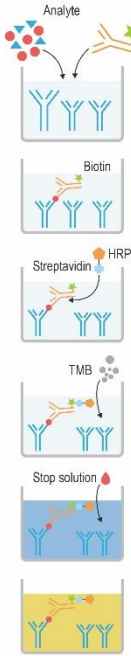
Samples were spiked with high concentrations of Human NGAL and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Urine(n=4)
1:2	Range (%)	88-99	90-101	101-110
	Average (%)	91	96	104
1:4	Range (%)	95-105	92-102	96-109
	Average (%)	99	97	102
1:8	Range (%)	87-96	86-100	98-111
	Average (%)	92	93	105
1:16	Range (%)	101-111	90-98	100-110
	Average (%)	105	95	104

Troubleshooting

Problem	causes	solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

SUMMARY



1. Add 50μL standard or sample to the wells, immediately add 50μL Biotinylated Detection Ab working solution to each well. Incubate for 90 min at 37°C
2. Aspirate and wash the plate for 3 times
3. Add 100μL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times
4. Add 90μL Substrate Reagent. Incubate for 15 min at 37°C
5. Add 50μL Stop Solution
6. Read the plate at 450nm immediately. Calculation of the results

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.