Human NGAL monomer-specific ELISA Kit

KIT 048

BioPorto® Diagnostics

For Research Use Only.
Not for use in diagnostic procedures.

Revision: NM2014-07RUO
Please read these instructions carefully.

APPLICATION

For the *in-vitro* determination of human NGAL monomer in urine, EDTA plasma and Li-Heparin plasma. For Research Use Only. Not for use in diagnostic procedures.

INTRODUCTION

NGAL (neutrophil gelatinase-associated lipocalin) was first discovered in 1989 and, as its name implies, belongs to the lipocalin family of proteins. These are typically small secreted proteins characterized by their ability to bind hydrophobic molecules in a structurally conserved pocket formed by β-pleated sheet, to bind to specific cell-surface receptors, and to form macromolecular complexes. NGAL was fully characterized and named in 1993, but has many synonyms: NL (neutrophil lipocalin; HNL: human NL), lipocalin 2, siderocalin, oncogene protein 24p3 or uterocalin (in the mouse) and *neu*-related lipocalin or 25-kDa α_2-microglobulin-related protein (in the rat). Human NGAL consists of a single disulfide-bridged polypeptide chain of 178 amino-acid residues with a calculated molecular mass of 21 kDa, but glycosylation increases its apparent molecular mass to 25 kDa. In neutrophils (neutrophilic polymorphonuclear leukocytes) it occurs in monomer and homodimer forms with a small percentage of higher molecular weight forms, and some of it is found in complex with 92-kDa human neutrophil type IV collagenase (gelatinase B or matrix metalloproteinase-9, MMP-9).

PRINCIPLE OF THE ASSAY

The assay is a sandwich ELISA performed in microwells coated with a monoclonal antibody to human NGAL. Bound NGAL is detected with another monoclonal NGAL antibody labeled with biotin and the assay is developed with horseradish peroxidase (HRP)-conjugated streptavidin followed by the addition of a color-forming substrate. The combination of antibodies is specific for human NGAL monomer and does not react with NGAL homodimers. The assay is a 4-step procedure:

Step 1. Aliquots of calibrators, diluted samples and any controls are incubated in microwells pre-coated with monoclonal capture antibody. NGAL present in the solutions will bind to the coat, while unbound material is removed by washing.

Step 2. Biotinylated monoclonal detection antibody is added to each test well and incubated. The detection antibody attaches to bound NGAL; unbound detection antibody is removed by washing.

Step 3. HRP-conjugated streptavidin is added to each test well and allowed to form a complex with the bound biotinylated antibody. Unbound conjugate is removed by washing.

Step 4. A color-forming peroxidase substrate containing tetramethylbenzidine (TMB) is added to each test well. The bound HRP-streptavidin reacts with the substrate to generate a colored product. The enzymatic reaction is stopped chemically, and the color intensity is read at 450 nm in an ELISA reader. The color intensity (absorbance) is a function of the concentration of NGAL originally added to each well. The results for the calibrators are used to construct a calibration curve from which the concentrations of NGAL in the test specimens are read.

KIT COMPONENTS

Item	Contents	Quantity
1	Microwell plate, 96 precoated wells	1 plate
2	5x Sample Diluent Conc.	1 x 50 mL
3	NGAL Calibrators. 0, 10, 25, 50, 100, 250, 500, 1000 pg/mL	8 x 1 mL
4	25x Wash Solution Conc.	1 x 40 mL
5	Biotinylated NGAL monomer-specific Antibody	1 x 12 mL
6	HRP-Streptavidin	1 x 12 mL
7	TMB Substrate	1 x 12 mL
8	Stop Solution	1 x 12 mL

Note: *Liquid reagents contain preservatives and may be harmful if ingested.*

MATERIALS REQUIRED BUT NOT PROVIDED

1. Adjustable micropipettes covering the range 1-1000 µL and corresponding disposable pipette tips
2. Polypropylene tubes to contain up to 1000 µL
3. Tube racks
4. Adjustable 8- or 12-channel micropipette (50-250 µL range) or repeating micropipette (optional)
5. Clean 1 L and 500 mL graduated cylinders
6. Deionized or distilled water
7. Cover for microwell plate
8. Clean container for diluted Wash Solution
9. Apparatus for filling wells during washing procedure (optional)
10. Lint-free paper towels or absorbent paper
11. Disposable pipetting reservoirs
12. Timer (60-minute range)
13. Calibrated ELISA plate reader capable of reading at 450 nm (preferably subtracting reference values at 650 or 620 nm)
14. Sodium hypochlorite (household bleach 1:10 dilution) for decontamination of specimens, reagents, and materials

PRECAUTIONS

For Research Use Only.
Not for use in diagnostic procedures

1. This kit should only be used by qualified laboratory staff.
2. Use separate pipette tips for each sample, calibrator and reagent to avoid cross-contamination.
3. Use separate reservoirs for each reagent. This applies especially to the TMB Substrate.
4. After use, decontaminate all specimens, reagents and materials by soaking for at least 30 minutes in sodium hypochlorite solution (household bleach diluted 1:10).
5. To avoid droplet formation during washing, aspirate the wash solution into a bottle containing bleach.
6. Avoid release into the environment. Dispose of containers and unused contents in a safe way and in accordance with national and local regulations.
7. The Stop Solution contains 0.5 M sulfuric acid and can cause irritation or burns to the skin and eyes. If contact occurs, rinse immediately with plenty of water and seek medical advice.
8. Do not interchange components from kits with different batch numbers. The components have been standardized as a unit for a given batch.
9. Hemolyzed, hyperlipemic, heat-treated or contaminated specimens may give erroneous results.
10. Do not dilute specimens directly in the microwells.
11. Do not touch or scrape the bottom of the microwells when pipetting or aspirating fluid.
12. Incubation times and temperatures other than those specified may give erroneous results.

13. Do not allow the wells to dry once the assay has begun.
14. The TMB Substrate is light sensitive. Keep away from bright light.
15. Do not reuse microwells or pour reagents back into their bottles once dispensed.

STABILITY AND STORAGE

1. Store the kit with all reagents at 2-8°C. Do not freeze.
2. Use all reagents before the expiry date on the kit box label.

COLLECTION OF SPECIMENS

Handle and dispose of all blood-derived or urine specimens as if they were potentially infectious. See Precautions, sections 1, 2, 4 and 5.

Determination of NGAL in a single specimen requires 10 µL of fluid sample. Blood specimens should be collected aseptically into heparinized or EDTA tubes by qualified staff using approved venipuncture techniques. Plasma should be prepared by standard techniques for laboratory testing. Urine should be centrifuged. Cap the prepared specimens and freeze them at −20°C or below. This especially applies if the assay cannot be performed within 24 hours or if the specimen is to be shipped. For long-term storage of specimens, −70°C or below is recommended. **Avoid repeated freezing and thawing.** Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens.

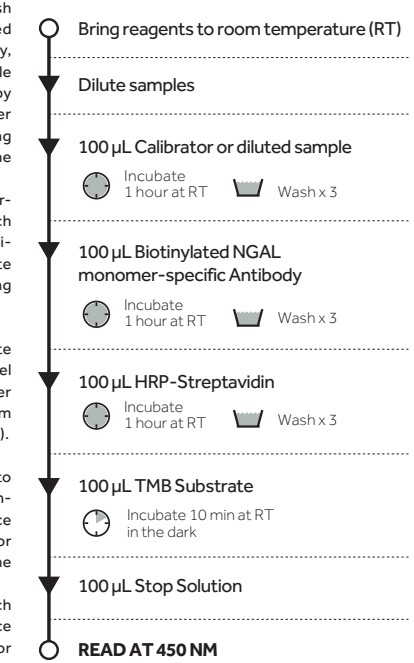
PREPARATION OF REAGENTS AND SAMPLES

1. Bring all specimens and reagents to room temperature (20-25°C). Mix specimens thoroughly by gentle inversion and if necessary clear visible particulate matter by low-speed centrifugation.
2. Wash Solution: Dilute the 25x Wash Solution Concentrate by pouring the total contents of the bottle (40 mL) into a 1-L graduated cylinder and add distilled or deionized water to a final volume of 1 L. Mix thoroughly.
3. Sample Diluent: Dilute the 5x Sample Diluent Concentrate (contains red dye to aid pipetting) by pouring the total contents of the bottle (50 mL) into a 250-mL graduated cylinder and add distilled or deionized water to a final volume of 250 mL. Mix thoroughly.
4. NGAL Calibrators (ready to use, contains red dye to aid pipetting): The assigned concentrations are indicated on their labels. Do not dilute further.
5. Biotinylated NGAL monomer-specific Antibody (ready to use): Do not dilute further.
6. HRP-Streptavidin Conjugate (ready to use): Do not dilute further.
7. TMB Substrate (ready to use): Do not dilute further.
8. Stop Solution (ready to use): Do not dilute further.
9. Specimens: Dilute each specimen in a recorded proportion with the pre-diluted Sample Diluent to obtain at least 250 µL of diluted solution that can be set up in duplicate wells at 100 µL per well. An initial screening at a dilution of 1/500 is recommended for physiological fluids. This can be prepared in two steps, as follows: dilute 10 µL of specimen in 190 µL of Sample Diluent to make a 1/20 dilution; then dilute 10 µL of the 1/20 dilution in 240 µL of Sample Diluent to make a 1/500 dilution. Dilutions are mixed by inversion or moderate vortexing. Out-of-range specimens should be re-assayed at lower or higher dilution as appropriate. Dilutions lower than 1/10 should not be used.

ASSAY PROCEDURE

1. Prepare the assay protocol, assigning the appropriate wells for setting up calibrators, diluted specimens and any internal laboratory controls in duplicate. If a reference wavelength of 650 or 620 nm is not available on the ELISA reader, a reagent blank well can be assigned. This is set up with 100 µL of Sample Diluent instead of diluted specimen and processed like the other wells.
2. Pipette 100 µL volumes of each calibrator, diluted specimens and any internal laboratory controls into the corresponding positions in the microwells. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform set at 200/minute.
3. Aspirate the contents of the microwells and wash the microwells three times with 300 µL diluted Wash Solution. If washing is done manually, empty the microwells by inversion and gentle shaking into a suitable container, followed by blotting in the inverted position on a paper towel. A dwell time of 1 minute before emptying is recommended for at least the last wash of the cycle.
4. Dispense 100 µL of Biotinylated NGAL monomer-specific Antibody (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (200/minute).
5. Wash as described above in Step 3.
6. Dispense 100 µL of HRP-Streptavidin Conjugate (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (200/minute).
7. Wash as described above in Step 3.
8. Dispense 100 µL of TMB Substrate (ready to use) into each microwell. The use of a multichannel micropipette is recommended to reduce pipetting time. Cover the wells and incubate for **exactly 10 minutes** at room temperature in the dark. Start the clock when filling the first well.
9. Add 100 µL Stop Solution (ready to use) to each well, maintaining the same pipetting sequence and rate as in Step 8. Mix by gentle shaking for 20 seconds, avoiding splashing. Read the wells within 30 minutes.
10. Read the absorbances of the wells at 450 nm in an appropriate microplate reader (reference wavelength 650 or 620 nm). If no reference wavelength is available, the value of the reagent blank well is subtracted from each of the other values before other calculations are performed.

SCHEMATIC OVERVIEW



CALCULATION OF RESULTS

A calibration curve is constructed by plotting the absorbance values obtained for the calibrators on the y-axis against the corresponding NGAL concentrations the x-axis. The calibration curve must meet the validation requirements. The NGAL concentrations of diluted samples are then found by placing their absorbance values on the calibration curve and reading the corresponding concentrations from the x-axis.

This procedure can be performed manually using graph paper with linear x and y scales. A smooth curve can be drawn through the points or adjacent points can be joined by straight lines. The latter procedure may slightly overestimate/underestimate concentration values between points when the curve is slightly convex to left/right, respectively. Although the curve may approximate to a straight line, it is both practically and theoretically incorrect to calculate and draw the straight line of best fit and to read the results from this.

The procedure can also be performed by an ELISA reader software program incorporating curve fitting procedures. The procedure of choice is to use linear x and y axes with 4-parameter logistic curve fitting. Diluted samples that give a mean absorbance above that for the 1000 pg/mL NGAL Calibrator or below that for the 10 pg/mL NGAL Calibrator are out of the range of the assay and their concentrations should be noted as >1000 pg/mL and <10 pg/mL respectively. The corresponding concentrations in the undiluted samples are calculated and reported as >(1000 x dilution factor) pg/mL and <(10 x dilution factor) pg/mL, respectively. If necessary, these samples can be re-assayed at higher and lower dilutions for high- and low-reading samples, respectively. The new dilution factors should be those estimated to give absorbance values that fall well within the range of the calibration curve, but dilutions lower than 1/10 should not be used.

VALIDATION OF CALIBRATION CURVE

The mean absorbance for the 1000 pg/mL NGAL Calibrator should be >1.5. The mean absorbance for any NGAL calibrator should be higher than that for the previous NGAL calibrator, e.g. absorbance (100 pg/mL NGAL) > absorbance (50 pg/mL NGAL). The curve should be slightly convex to the left when the results are plotted on linear axes.

CALIBRATION TROUBLESHOOTING

Out-of-line points for individual calibrators: One or more individual calibrators may give anomalous absorbance readings. One or both of the duplicate values may be out of line, and the mean of the duplicates may be out of line. This error is significant if it impairs satisfactory curve fitting by the 4-parameter logistic method, which, as a result of the anomalous value, is shifted away from other calibrator points that are in fact correct. The calibrator points and fitted curve should always be examined for correct fit before any calculations of concentration from it are accepted. A poorly fitting curve will also be revealed by a high sum of residual squares. If only one calibrator is affected, which is not the highest calibrator, two courses of action are possible:

i) An erroneous singlet or duplicate result should be eliminated from the curve, and the remaining results refitted by the 4-parameter logistic procedure. If a satisfactory fit is obtained, provisional concentration results can be calculated from it.

ii) If no satisfactory fit can be obtained in this way, but the curve is otherwise consistent, provisional results can be obtained from straight lines or simple cubic spline fitting between the means of duplicates, omitting the erroneous point.

If two or more calibrators are affected, the assay should be repeated.

A deviant result for an individual calibrator can be due to operator error or to calibrator deterioration. If both duplicate values are consistently out of line in successive assays, the calibrator is faulty and should be omitted.

TRACEABILITY OF CALIBRATOR VALUE

No internationally approved reference material for NGAL is currently available. The NGAL concentration of calibrator material has been assigned by turbidimetry using a precise transfer protocol ensuring traceability to the BioPorto Diagnostics master calibrator. The reference material was value assigned by measurement of light absorbance at 280 nm using a theoretically calculated extinction coefficient based on the amino-acid composition

QUALITY CONTROL

Laboratories intending to perform repeated assays should establish their own high-reading and low-reading control sera or urine, stored in small (e.g. 50 µL) aliquots at –70°C or below.

An aliquot of each should be thawed and tested in each assay and a record kept of successive results. This serves as a control of test performance, test integrity and operator reliability.

The results should be examined for drift (tendency for successive results to rise or fall) or significant deviation from the mean of previous results. Values not deviating by more than 20% from the mean of previous values can be taken to indicate acceptability of the assay.

Aliquots of control specimens should not be refrozen for repeated assay once thawed, and if a further assay is performed, fresh control aliquots and fresh dilutions of specimens should be used.

LIABILITY

This ELISA Kit is only intended for the in vitro determination of human monomer-specific NGAL in fluid samples. The ELISA Kit is only intended for use by qualified personnel carrying out research activities. If the recipient of this test passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at recipient's own risk secure in favor of BioPorto Diagnostics A/S all limitations of liability herein.

Catalogue number

Batch code


Consult instructions for use


Use by


Manufacturer


Keep away from sunlight


Temperature limitation


Do not reuse


Caution, consult accompanying documents


Biological risk


Do not use if package is damaged

Concentrated Sample Diluent. Dilute before use.

Concentrated Wash Solution. Dilute before use.

RELATED PRODUCTS

Cat. No.	Product name
KIT 036	NGAL ELISA Kit
KIT 042	Mouse NGAL ELISA Kit
KIT 043	Dog NGAL ELISA Kit
KIT 044	Pig NGAL ELISA Kit
KIT 045	Monkey NGAL ELISA Kit
KIT 046	Rat NGAL ELISA Kit

BioPorto® Diagnostics

BioPorto Diagnostics A/S, Tuborg Havnevej 15, st., DK-2900 Hellerup
Phone (+45) 4529 0000, info@bioporto.com, www.bioporto.com

50901/082014/e