NGAL Rapid ELISA Kit

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

KIT 037

Bioporto®
Diagnostics
Please read these instructions carefully

APPLICATION
The NGAL Rapid ELISA Kit measures the amount of human NGAL in tissue fluids (e.g. plasma, serum or urine), tissue extracts or culture media. For research use only. Not for use in diagnostic procedures.

INTRODUCTION
NGAL1 (neutrophil gelatinase-associated lipocalin) belongs to the lipocalin family of proteins. These are typically small secreted proteins characterized by their ability to bind small, 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 has many synonyms: it is also known as NL (neutrophil lipocalin; HNL: human NL)2, lipocalin 2, oncogene protein 24p3 3 or uterocalin 4 (in the mouse) and neu-related lipocalin 5 or 25 kDa α2-microglobulin-related protein 6 (in the rat). Human NGAL consists of a single disulfide-bridged polypeptide chain of 178 amino-acid residues with a calculated molecular mass of 22 kDa, but glycosylation increases its apparent molecular mass to 25 kDa. In neutrophils (neutrophil polymorphonuclear leukocytes) and urine it occurs as monomer, with a small percentage of dimer and trimer, and also in complex with 92-kDa human neutrophil type IV collagenase, also called gelatinase B or matrix metalloproteinase-9 (MMP-9)7.

NGAL was originally isolated from the supernatant of activated human neutrophils3, but it is also expressed at a low level in other human tissues including the kidney, prostate and epithelia of the respiratory and alimentary tracts8,9. It is strongly expressed in adenomas and inflamed epithelia of the bowel10, adenocarcinomas of the breast11, and urothelial carcinomas12.

Because of its small molecular size and resistance to degradation, NGAL is readily excreted and detected in the urine, both in its free form and in complex with MMP-9. Urinary levels correlate with plasma or serum levels whatever the cause of increased NGAL production (BioPorto Diagnostics data), but particularly high urinary levels can be expected when NGAL is released directly into the urine by the kidney tubules or urothelial carcinomas. It is uncertain how far NGAL-MMP-9 complexes from sources remote from the urinary tract are excreted as such into the urine or reform in the urine after independent excretion of NGAL and MMP-97.

While the functions of NGAL are not fully understood, NGAL appears to be upregulated in cells under “stress”, e.g. from infection, inflammation, ischemia or neoplastic transformation, or in tissues undergoing involution, such as the postpartum mouse uterus and mammary glands on weaning. In relation to a possible antibacterial role, it binds enterobactin and other siderophores, depriving the microorganisms of Fe3+, an important nutritional requirement13. Its complex formation with MMP-9 appears to protect MMP-9 enzymatic activity from degradation7. The upregulation of NGAL in involuting tissues has led to the postulation of a role in apoptosis, but it appears more likely that NGAL is associated with a survival response14. This seems to be so in the kidney, where NGAL-siderophore-iron complex rescues the mouse kidney from ischemic injury15.

NGAL and the kidney. Even before NGAL had been isolated from human neutrophils, its mouse homologue 24p3 was known to be expressed by kidney cells and to undergo an early, dramatic upregulation (14- to 20-fold) in response to SV 40 viral infection16. A similar early and dramatic upregulation was later observed in rat proximal tubule cells after ischemia-reperfusion injury17, and raised plasma levels of NGAL were found to be strongly correlated with decreased renal function in patients with renal damage due to systemic vasculitis18. The results for renal ischemia-reperfusion injury were subsequently confirmed and extended to nephrotoxic agents19,20,21. It has been suggested that urinary NGAL levels may serve as an early marker for ischemic renal injury in children after cardiopulmonary bypass22. Raised urinary and serum NGAL levels have also been observed in patients with established renal failure (BioPorto Diagnostics data) and patients with functioning renal grafts also
NGAL Rapid ELISA Kit

showed raised urinary levels (detectable by Western blotting)\(^2\). It is therefore apparent that a large variety of renal disorders are associated with raised plasma and urinary levels of NGAL. While plasma and urinary NGAL levels are closely correlated in acute conditions, it is to be expected that urinary NGAL levels will be particularly high after ischemic renal injury severe enough to result in acute renal failure, acute tubular necrosis or acute tubulo-interstitial nephropathy. However, the use of urinary NGAL as a marker for these conditions is subject to the proviso that other concurrent conditions that are independently associated with raised NGAL levels must be taken into account.

**NGAL in inflammation/infection.** NGAL is released from the secondary granules of activated neutrophils\(^1\) and plasma levels rise in inflammatory or infective conditions, especially in bacterial infections\(^2,3\). Thus the level of NGAL in plasma or serum has been proposed as a marker of infection. However, as levels of NGAL may also be raised in neoplastic conditions and renal disorders independently of any infective process, this proposed application should be treated with caution. NGAL may also be raised in infections in patients with an uncountably low number of neutrophils due to leukemia or treated leukemia, showing that the source of the raised NGAL in infections is not only the neutrophils. Indeed, serum NGAL levels correlate very poorly with the neutrophil count in patients with varying degrees of infection or inflammation (BioPorto Diagnostics data).

**NGAL and neoplasia.** The various types of cancer in which NGAL may be upregulated (often with MMP-9) have been referred to above. This has been shown by its expression in tumor cells and its high urinary levels, both in the free form and complexed with MMP-9\(^7\). Indeed, it has been proposed that urinary NGAL-MMP-9 complexes may serve as a marker of disease status for breast cancer patients\(^24\). Plasma levels have not usually been measured in these cases.

**NGAL as a potential diagnostic marker.** The finding of a raised urinary or plasma level of NGAL cannot be independently diagnostic of any single pathology. As stated above, a variety of independently associated with raised levels of urinary or plasma NGAL. For this reason the present kit is presented for research use only.
PRINCIPLE OF THE ASSAY PROCEDURE

The assay is an ELISA performed in microwells coated with a monoclonal antibody against human NGAL. Bound NGAL is detected with a horseradish peroxidase (HRP)-conjugated monoclonal antibody and the assay is developed by incubation with a color-forming substrate. The assay is a rapid 2-step procedure:

**Step 1.** Aliquots of calibrators, diluted samples and any controls are incubated with HRP-conjugated detection antibody in the coated microwells. Only NGAL will bind to both coat and detection antibody, while unbound materials are removed by washing.

**Step 2.** A chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) is added to each test well. The HRP linked to the bound detection antibody 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.

**NGAL antibody**
Plates are precoated with the primary NGAL antibody. Plates are ready to use.

**HRP-conjugated NGAL antibody**
HRP-conjugated detection antibody is added to each well.

**Calibrators and diluted samples**
Calibrators and diluted samples are added to the wells and incubated.

**TMB Substrate**
Substrate is added to each well and developed.

**Stop Solution**
Stop Solution is added to each well. Quantitative results are obtained by measuring the absorbance at 450 nm.
KIT COMPONENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Contents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 x 8 coated Microwells + Frame</td>
<td>96 wells</td>
</tr>
<tr>
<td>2</td>
<td>5x Sample Diluent Conc.</td>
<td>1 x 60 mL</td>
</tr>
<tr>
<td>2</td>
<td>NGAL Rapid Calibrator 1-6</td>
<td>6 x 1 mL</td>
</tr>
<tr>
<td>2</td>
<td>The exact concentration of each calibrator is printed on the label of the vials and on the QC-certificate.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25x Wash Solution Conc.</td>
<td>1 x 30 mL</td>
</tr>
<tr>
<td>4</td>
<td>HRP-conjugated NGAL Antibody</td>
<td>1 x 6 mL</td>
</tr>
<tr>
<td>5</td>
<td>TMB Substrate</td>
<td>1 x 12 mL</td>
</tr>
<tr>
<td>6</td>
<td>Stop Solution</td>
<td>1 x 16 mL</td>
</tr>
<tr>
<td>7</td>
<td>Polypropylene U-Microwell Plate</td>
<td>96 wells</td>
</tr>
</tbody>
</table>

Note: Liquid reagents contain the preservatives sodium azide, thimerosal or Bronidox L. These 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 microplate
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 5x Sample Diluent Concentrate is preserved with 0.25% sodium azide (corresponding to 0.05% in final diluted solution). Sodium azide is harmful in contact with skin and if swallowed. Sodium azide is harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). According to EU regulations, no danger labeling is necessary for the diluted solution.
8. The Stop Solution contains 0.5 mol/L 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.
9. Do not interchange components from kits with different batch numbers. The components have been standardized as a unit for a given batch.
10. Hemolyzed, hyperlipemic, heat-treated or contaminated specimens may give erroneous results.
11. Do not dilute specimens directly in the coated microwells.
12. Do not touch or scrape the bottom of the coated microwells when pipetting or aspirating fluid.
13. Incubation times and temperatures other than those specified may give erroneous results.
14. Do not allow the wells to dry once the assay has begun.
15. The TMB Substrate is light sensitive. Keep away from bright light.
16. 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 vial labels.
3. Diluted Wash Solution Concentrate remains stable for 4 weeks at 2-8°C. If not all wells are to be used, dilute only the portion of Wash Solution Concentrate required.
4. Diluted Sample Diluent Concentrate remains stable for 5 days at 2-8°C. If not all wells are to be used, dilute only the portion of Sample Diluent Concentrate required.
5. For subsequent use, store unused wells in the foil pouch with the desiccant provided and reseal. Always allow foil pouch to equilibrate to room temperature before opening to avoid condensation in/on the coated microwells.

COLLECTION OF SPECIMENS
Handle and dispose of all blood-derived or urine specimens as if they were potentially infectious. See Precautions, sections 2, 4 and 5.
Determination of NGAL in a single specimen requires 10 µL of urine, plasma or serum. Blood specimens should be collected aseptically into EDTA tubes or plain tubes by qualified staff using approved venepuncture techniques. Plasma or serum should be prepared by standard techniques for clinical laboratory testing. Urine should be centrifuged. Cap the prepared specimens. If the assay cannot be performed within 24 hours or specimens are to be shipped, freeze the specimens at −20°C or below. 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
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. Determine the number of specimens to be tested (in duplicate) plus any internal laboratory control specimens (in duplicate) plus any reagent blank wells. The precoated wells can be used as strips of 8 or as individual wells. Single wells are handled by breaking individual wells apart and placing each well in the frame at an appropriate position. Letters and notches on the wells allow the individual wells to be identified. Add 12 wells for the 6 calibrators (in duplicate). Remove the number of microwells required and replace the remainder in the foil pouch with desiccant at 2-8°C.
3. Wash Solution: Dilute the 25x Wash Solution Concentrate by pouring the total contents of the bottle (30 mL) into a 1-L graduated cylinder and add distilled or deionized water to a final volume of 750 mL. Mix thoroughly and store at 2-8°C. If not all the wells are to be used, dilute only the required volume of Wash Solution Concentrate with 24 volumes of water to produce a 1/25 dilution.
4. Sample Diluent: Dilute the 5x Sample Diluent Concentrate (contains yellow dye to aid pipetting) by pouring the total contents of the bottle (60 mL) into a 500-mL graduated cylinder and add distilled or deionized water to a final volume of 300 mL. Mix thoroughly and store at 2-8°C after use. If not all the wells are to be used, dilute only the required volume of Sample Diluent Concentrate with 4 volumes of water to produce a 1/5 dilution.
5. NGAL Rapid Calibrators (ready to use): The assigned concentrations are indicated on their labels. Do not dilute further.
6. HRP-conjugated NGAL Antibody (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.

PREPARATION OF SAMPLES
The approximate range of the standard curve is 0.2-20 ng/mL and the diagnostically relevant range is 100-500 ng/mL for plasma or serum and 50-500 ng/mL for urine (see Interpretation of Results). Therefore an initial screening dilution of 1/100 for plasma or serum and 1/50 for urine is recommended. The 1/100 dilution can be prepared by diluting 10 µL of plasma or serum in 990 µL of Sample Diluent and the 1/50 dilution can be prepared by diluting 10 µL of urine in 490 µL of Sample Diluent. Dilutions are mixed by inversion or moderate vortexing. Reassay of out-of-range samples at lower or higher dilution may be necessary. Dilutions lower than 1/25 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 50 µL of Sample Diluent instead of diluted sample and processed like the other wells.
2. Dilute samples according to the expected NGAL concentrations (1/100 for plasma or serum and 1/50 for urine will be suitable for most samples).
3. Pipette a sufficient volume of each calibrator, each diluted sample and any internal laboratory controls into the appropriate wells of the polypropylene U-microwell plate to permit subsequent transfer of 50 µL volumes to corresponding coated microwells.
4. Pipette 50 µL volumes of HRP-conjugated NGAL Antibody into the corresponding positions in the coated microwells. Then with a multichannel pipette rapidly transfer 50 µL volumes of the calibrator solutions, diluted samples and internal controls from the U-wells into the corresponding coated wells already containing the detection antibody. This method of sample addition is recommended to reduce the difference in incubation time between the first and last samples added to the coated microwells.
5. Cover the wells and incubate for 30 minutes at room temperature on a shaking platform set at 200/minute.
6. Aspirate the contents of the microwells and wash the microwells three times with at least 300 µL of the previously diluted Wash Solution. If washing is performed 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. The vigor with which diluted Wash Solution is filled into or emptied from the wells influences final color development. Manual pipetting, which may be very gentle and lead to high color development, is only recommended in the absence of alternatives such as filling the wells by immersion, using a multi-channel manual washing dispenser, or using an automatic washing apparatus.
7. 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 15 minutes at room temperature in the dark. Start the clock when filling the first well.
8. Add 100 µL of Stop Solution (ready to use) to each well, maintaining the same pipetting sequence and rate as in Step 7. Mix by gentle shaking for 20 seconds, avoiding splashing. Read the wells within 30 minutes.
9. Read the absorbances of the wells at 450 nm in an appropriate microplate reader (reference wave-length 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**

1. **Bring reagents to RT**
2. **Dilute samples**
3. **50 μL HRP-conjugated NGAL Antibody + 50 μL Calibrator or diluted sample**
   - Incubate 30 min at RT
   - Wash x 3
4. **100 μL TMB Substrate**
   - Incubate 15 min at RT in the dark
5. **100 μL Stop Solution**
6. **Read at 450 nm**

**CALCULATION OF RESULTS**

For calculation of results the exact values of the calibrators printed on the labels of the calibrators and on the QC-certificate should be used.

The basic principle is to construct a calibration curve by plotting the mean of duplicate absorbance values for each NGAL Rapid Calibrator on the y-axis against the corresponding NGAL concentrations in ng/mL on the x-axis. The calibration curve must meet the validation requirements. The NGAL concentration of each diluted sample is then found by locating the point on the curve corresponding to the mean of duplicate absorbance values for the diluted sample and reading its corresponding concentration in ng/mL from the x-axis. The concentration of NGAL in the undiluted specimen is calculated by multiplying this result by the sample dilution factor.

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 concentration values between points when the curve is slightly convex to the left, which is the typical finding. 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 NGAL Rapid Calibrator 6 or below that for the NGAL Rapid Calibrator 2 are out of the range of the assay and their concentrations should be noted as >A ng/mL and <B ng/mL, respectively, where A is the exact concentration of NGAL Rapid Calibrator 6 and B is the exact concentration of NGAL Rapid Calibrator 2. The corresponding concentrations in the undiluted sera are calculated > (A x dilution factor) ng/mL and < (B x dilution factor) ng/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 1/25 should not be used.

VALIDATION OF CALIBRATION CURVE

The mean absorbance for the NGAL Rapid Calibrator 6 should be >1.5. The mean absorbance for any NGAL Rapid Calibrator should be higher than that for the previous NGAL Rapid calibrator, e.g. absorbance(NGAL Rapid Calibrator 6) > absorbance(NGAL Rapid Calibrator 5). The curve should be slightly convex to the left when the results are plotted on linear axes.

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

The NGAL concentration of diluted calibrator material has been assigned by comparison in ELISA with a purified preparation of recombinant human NGAL quantified by Rigshospitalet, Copenhagen, Denmark.

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 serum 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.

OBSERVED RESULTS

The mean NGAL concentration in samples from healthy donors was 63 ng/mL (range 37–106 ng/mL, n = 80) in EDTA plasma and 5.3 ng/mL (range 0.7–9.8 ng/mL, n = 7) in urine. In unselected patients admitted to intensive care, the NGAL concentrations in urine ranged from 9 ng/mL to 40,000 ng/mL (40 µg/mL) in urine (n = 60) and from 25 ng/mL to 3491 ng/mL in EDTA plasma (n = 60).

PERFORMANCE CHARACTERISTICS

Limit of detection: The lowest concentration of NGAL giving an absorbance reading greater than 2 SD above the mean zero (NGAL Rapid Calibrator 1)
reading (n = 6) was determined 4 times by different operators. The results were <0.1 ng/mL, these being lower than the value of NGAL Rapid Calibrator 2.

**Precision:** Intraassay variation was determined by measurement of NGAL in a urine sample and a serum sample with 6 replicates in 4 separate assays performed by 4 different operators. The following results were obtained (CV = coefficient of variation):

<table>
<thead>
<tr>
<th>Samples</th>
<th>CV Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>3.4% (2.5-8.4)</td>
</tr>
<tr>
<td>Serum</td>
<td>3.2% (1.5-5.4)</td>
</tr>
</tbody>
</table>

Interassay variation was determined by measurement of NGAL in 7 diluted urine samples, 6 diluted serum and 7 diluted EDTA plasma samples with 2 replicates in 4 separate assays, performed by 4 different operators. The following results were obtained:

<table>
<thead>
<tr>
<th>Samples</th>
<th>CV Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>12.6% (5.7-30.4)</td>
</tr>
<tr>
<td>Serum</td>
<td>7.4% (4.9-10.5)</td>
</tr>
<tr>
<td>EDTA plasma</td>
<td>13.5% (6.5-22.3)</td>
</tr>
</tbody>
</table>

**Analytical recovery:** Urine and plasma samples were spiked with recombinant human NGAL and analyzed in the assay. Recovery was calculated from (Measured/Expected) expressed as a percentage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured</th>
<th>Expected</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>3.4 ng/mL</td>
<td>3.5 ng/mL</td>
<td>96%</td>
</tr>
<tr>
<td>Plasma</td>
<td>5.4 ng/mL</td>
<td>6.2 ng/mL</td>
<td>85%</td>
</tr>
</tbody>
</table>

**Linearity:** NGAL was measured in serial dilutions (n = 6) of a urine sample and a serum sample in 4 separate assays performed by 4 different operators. The CV of the mean of the measured values corrected for the dilution was 7.0% for urine and 8.5% for serum, demonstrating satisfactory linearity.

**Sample material:** Analysis of samples of serum, EDTA plasma or urine showed no significant differences in analytical recovery, linearity or precision. However, NGAL concentrations are often slightly higher in serum than in plasma, probably because of the release of NGAL from neutrophils during blood clotting.

**Specificity:** The two monoclonal antibodies against human NGAL used in the assay have been shown to bind to different preparations of recombinant human NGAL and to give a single band at 25 kDa on Western blot analysis of a reduced postnuclear supernatant from human neutrophils.

**LIABILITY**

*For Research Use Only.*

*Not for use in diagnostic procedures.*

This kit is only intended for the *in vitro* determination of NGAL in human plasma, serum or urine.

The kit is only intended for use by qualified personnel carrying out research activities.

If the recipient of this kit passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at own risk secure in favor of BioPorto Diagnostics A/S all limitations of liability herein.

BioPorto Diagnostics A/S shall not be responsible for any damages or loses due to using the kit in any way other than as expressly stated in these Instructions.

The liability of BioPorto Diagnostics A/S shall in no event exceed the commercial value of the kit.

BioPorto Diagnostics A/S shall under no circumstances be liable for indirect, special or consequential damages, including but not limited to loss of profit.

**Revision:** NR2009-04RUO
REFERENCES


NGAL Rapid ELISA Kit

Catalogue number
Référence du catalogue
Número de catálogo

Batch code
Code du lot
Código de lote

Consult instructions for use
Consulter les instructions d'utilisation
Consulte las instrucciones de uso

Use by
Utiliser jusque
Fecha de caducidad

Manufacturer
Fabricant
Fabricante

Keep away from sunlight
Ne pas exposer aux rayons solaires
Mantener fuera de la luz solar

Temperature limitation
Limites de température
Límite de temperatura

Concentrated Wash Solution.
Dilute before use.
Solution de bain concentrée.
Diluer avant usage.
Tampón de lavado concentrado.
Diluir antes de usar.

Concentrated Sample Diluent.
Dilute before use.
Diluant échantillon concentrée.
Diluer avant usage.
Tampón de dilución concentrado.
Diluir antes de usar.

Caution, consult accompanying documents
Attention, voir notice d'instructions
Atención, ver instrucciones de uso

Biological risk
Risques biologiques
Riesgo biológico

Do not use if package is damaged
Ne pas utiliser si l'emballage est endommagé
No usar si el paquete está dañado

Do not reuse
Ne pas réutiliser
No reutilizar