

# TechNotes

Clinical and  
Research Area

Kidney  
Diseases

Inflammation



## Neutrophil gelatinase-associated lipocalin (NGAL)



**N**eutrophil gelatinase-associated lipocalin (NGAL), which is also known as Lipocalin-2, is a small glycoprotein that belongs to the lipocalin superfamily. Whilst it is found in neutrophil secondary granules, several cell types express NGAL in response to injuries (1). The function of NGAL is not yet fully understood.

However, it has been shown to be involved in innate immunity response by binding to siderophores produced by bacteria. Siderophores carry iron that is needed by bacteria in order to grow and by limiting the available iron, NGAL can limit bacterial growth (2).

NGAL can be detected in the blood and urine in three different forms: a 25-kDa monomer, a 45-kDa disulfide-linked homodimer, and a 135-kDa heterodimer. The heteromeric form consists of covalently bound NGAL and matrix metalloproteinase 9 (MMP-9) (3).

### NGAL in diagnostics

NGAL has been indicated as a promising marker of **acute kidney injury (AKI)** (4). AKI causes an abrupt decline in renal function and it is defined as a minimum increase of serum creatinine by 0.3 mg/dl in 48 hours or by a decrease in urine output (<0.5 ml/kg/h for six hours) (5). NGAL is produced by kidney tubular epithelial cells and its levels rapidly increase after tubular damage. In fact, NGAL levels have been shown to increase within just two hours following an injury (6) and it would therefore allow for a faster detection

### CLINICAL UTILITY

✓ Acute kidney injury (AKI)

of AKI than serum creatinine (7,8). The use of NGAL, along with a panel of other renal biomarkers, could help to improve the early detection of AKI. In Europe, a few NGAL tests have been approved for clinical use. NGAL may also be involved in the pathophysiological process of chronic kidney diseases (CKD). Further, NGAL levels correlate with the severity of renal impairment (9). Therefore, NGAL is a direct marker of structural kidney injury and it could serve to either identify early renal damage or predict renal disease progression.

In addition to its promising role in the detection of AKI, NGAL has been indicated as a **nonspecific marker of acute infections** — similar to that of C-reactive protein and PCT, for example (10). The main form of NGAL that correlates with the inflammation appears to be the homodimeric NGAL, which is the form that is primarily found in neutrophil granules. .

### Reagents for assay development

At HyTest, we provide several mouse and rabbit-derived monoclonal antibodies that are specific to human NGAL and which are suitable for assay development. In addition, we offer recombinant NGAL that can be used as a calibrator or standard in NGAL immunoassays, or for biochemical and immunochemical studies of NGAL.

## Monoclonal antibodies specific to NGAL

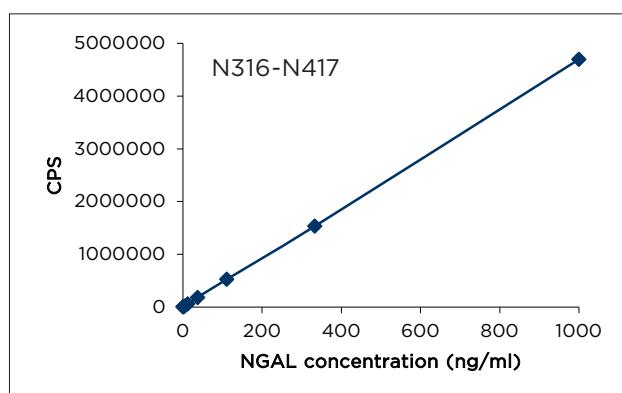
Our antibodies display different specificities towards the various forms of NGAL that are found in blood (see Table 1). For the development of sandwich immunoassays, we recommend several different pairs (see Table 2). A calibration curve for recombinant NGAL using the N316-N417 assay is shown in Figure 1. The pair N316-N417 as well as N308-N432 (not shown) demonstrate a broad dynamic range in our two-step sandwich fluoroimmunoassay (1-1000 ng/ml).

**Table 1. Specificities of anti-NGAL antibodies.**

MAb	Monomer	Homodimer	Heterodimer
N308	•	•	
N316	•	•	•
N417	•	•	•
N422	•	•	•
N432	•	•	
N457	•	•	
N461	•	•	•

**Table 2. Recommended pairs for the detection of different forms of NGAL using a sandwich immunoassay.**

Capture	Detection	Forms detected		
		Monomer	Homodimer	Heterodimer
N316	N417	•	•	•
N316	N461	•	•	•
N422	N417	•	•	•
N422	N461	•	•	•
N308	N432	•	•	
N316	N457		•	



**Figure 1. The calibration curve for MAb pair N316-N417.**

The detection antibody was labelled with Eu<sup>3+</sup>. Recombinant NGAL (Cat.# 8NL2) was used as the antigen. The pair shows a wide linearity range.

## Detection of NGAL in plasma samples

We measured NGAL in plasma samples from 29 patients with kidney disease and 14 apparently healthy individuals with two prototype assays N308-N432 and N316-N417. The results show a severalfold difference between the two groups in mean concentration of NGAL (see Figure 2).

In addition, we compared the same prototype assays with a commercially available NGAL kit that is intended for in vitro diagnostics (Human NGAL ELISA (Kit 036CE) from BioPorto). With our in-house assay, our recombinant NGAL was used as the calibrator. Furthermore, the BioPorto kit was used according to the manufacturer's instructions. Our preliminary correlation studies that were made with a limited sample set (featuring plasma samples from 18 human beings diagnosed with acute kidney injury, chronic kidney injury, or a non-kidney disease) showed a good to moderate correlation with the Spearman Rank Order Correlation (see Table 3).

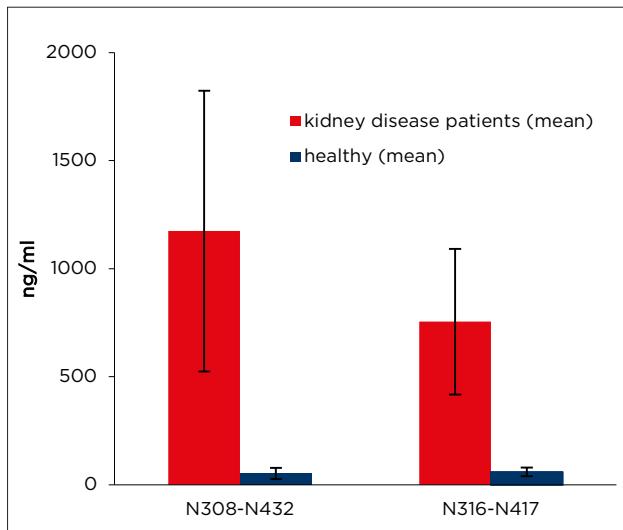


## RABBIT RECOMBINANT MONOCLONAL ANTIBODIES FROM HYTEST

HyTest has developed a new monoclonal antibody technology that is based on rabbit-derived antibodies. One of the company's core strengths is its considerable experience (25+ years) in hybridoma monoclonal antibody development. The new technology combines the reliability of the hybridoma approach with rapid and flexible gene engineering methods. Our rabbit recombinant antibody technology is based on the natural immune response of rabbits and it includes a robust proprietary protocol for the cloning of target IgG genes to full-size rabbit antibody backbone.

Recombinant rabbit MAbs will be available in bulk quantities (gram scale), which makes them suitable for commercial diagnostic immunoassays.

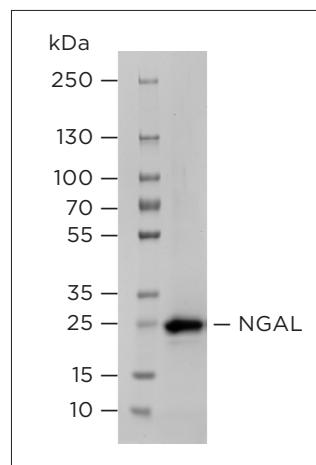
Combining a recombinant rabbit MAb with a conventional mouse-derived MAb in sandwich type immunoassays also helps to mitigate the effect of heterophile antibodies.



**Figure 2. The mean concentrations of NGAL measured in patients who were diagnosed with a kidney disease and in apparently healthy individuals.** The NGAL concentration in patients with a kidney disease was several fold higher than in healthy individuals when measured using two HyTest prototype assays: N308-N432 and N316-N417. N=29 for patients with a kidney disease, N=14 for healthy individuals.

### Recombinant human NGAL

HyTest offers a recombinant NGAL antigen that can be used as a calibrator in immunoassays and in NGAL biochemical and immunochemical studies. The antigen is produced in a mammalian cell line and it contains a C-terminal His-tag. Recombinant NGAL is purified to near homogeneity using chromatographic techniques (see Figure 3).



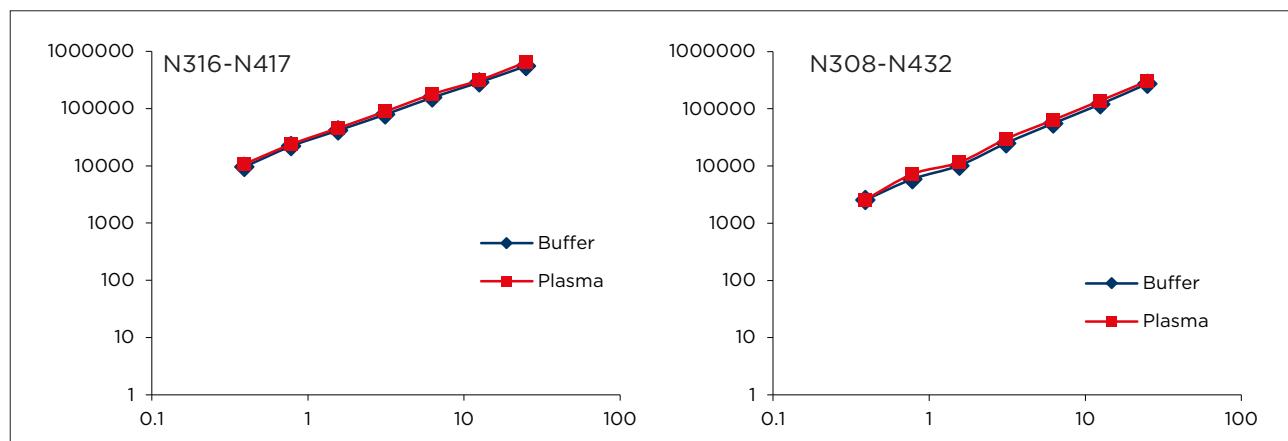
**Figure 3. SDS-PAGE of recombinant NGAL under reducing conditions in a gradient gel (4-20%).** 4 µg of purified protein was loaded on the gel.

**Table 3. The Spearman Rank Order Correlations for two HyTest prototype assays and Human NGAL ELISA (Kit 036CE) from BioPorto.** Marked correlations are significant at  $p < 0.05000$ .

Assay	N308-N432	N316-N417
Human NGAL ELISA	0.77	0.97

### Parallel calibration curves for native and recombinant NGAL

We examined the immunochemical properties of recombinant NGAL in comparison to endogenous NGAL in plasma samples. As shown in Figure 3, the calibration curve for the recombinant NGAL was parallel to that of native NGAL. This indicates that the recombinant NGAL could be used as a calibrator in NGAL assays.



**Figure 4. The titration curves for recombinant NGAL and native NGAL in human plasma using the MAb pairs N316-N417 (A) and N308-N432 (B).**

## Ordering information

### MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Neutrophil gelatinase-associated lipocalin (NGAL)	4NG7	N308	IgG	EIA, WB, recombinant rabbit antibody
		N316	IgG	EIA, WB, recombinant rabbit antibody
		N417	IgG1	<i>In vitro</i> , EIA, WB
		N422	IgG1	<i>In vitro</i> , EIA
		N432	IgG1	<i>In vitro</i> , EIA
		N457	IgG1	<i>In vitro</i> , EIA
		N461	IgG1	<i>In vitro</i> , EIA

### ANTIGEN

Product name	Cat. #	Purity	Source
Neutrophil gelatinase-associated lipocalin (NGAL), human, recombinant	8NL2	>90%	Recombinant

### References

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2. **Flo, TH et al.** Lipocalin2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004 Dec;432(7019):917-21.
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4. **Soni, SS et al.** NGAL: a biomarker of acute kidney injury and other systemic conditions. Int Urol Nephrol. 2010 Mar;42(1):141-50.
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8. **Zappitelli, M et al.** Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. Crit Care. 2007;11(4):R84.
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10. **Venge, P.** Human neutrophil lipocalin (HNL) as a biomarker of acute infections. Ups J Med Sci. 2018 Jan 2;123(1):1-8.