Advanced ImmunoChemical Inc.

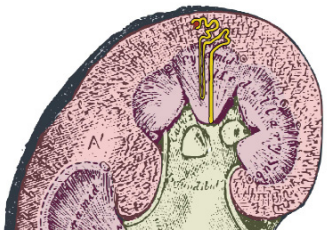
Providing for the Advancement of Clinical Diagnostics

ASSAY NOTES

Product Information

Courtesy of HyTest, Ltd

Neutrophil gelatinase-associated lipocalin (NGAL)



NGAL (neutrophil gelatinase-associated lipocalin, also known as Lipocalin-2) is a small glycoprotein that belongs to the lipocalin superfamily. It is found in neutrophil secondary granules, however, several cell types express NGAL in response to injuries (1). The function of NGAL is not fully understood, however, it has been shown to be involved in innate immunity response by binding to siderophores produced by bacteria. Siderophores carry iron needed by bacteria to grow and by limiting the available iron NGAL can limit bacterial growth (2).

NGAL can be detected in blood and urine in three different forms: a 25-kDa monomer, a 45-kDa disulphide-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 by increase in serum creatinine within 48 hours or by decrease in urine output in six hours (5). NGAL is produced by kidney tubular epithelial cells and its levels rapidly increase after tubular damage. NGAL levels have been shown to increase in just two hours after the injury (6) and thus it would allow for a faster detection of AKI than serum creatinine (7,8). The use of NGAL along with a panel of other renal biomarkers could improve the early detection of AKI. In Europe, a few NGAL tests have been approved for clinical use.

CLINICAL UTILITY

- **Acute kidney injury (AKI) early damage**
- **Monitor renal disease progression**
- **Non-specific marker of acute infections**

NGAL may also be involved in the pathophysiological process of chronic kidney diseases (CKD). NGAL levels correlate with severity of renal impairment (9). Thus, NGAL is a direct marker of structural kidney injury and could serve to 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** — similarly to that of C-reactive protein and PCT, for example (10). The main form of NGAL that correlates with the in ammation appears to be the homodimeric NGAL. Homodimeric NGAL is the form primarily found in neutrophil granules.

Reagents for assay development

We provide several mouse and rabbit derived monoclonal antibodies specific to human NGAL and 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 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 N316-N417 assay is shown in Figure 1. Pair N316-N417 as well as N308-N432 (not shown) show a broad dynamic range in our two-step sandwich fluoroimmunoassay (1-1000 ng/ml).

Table 1. Specifiities 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		•	

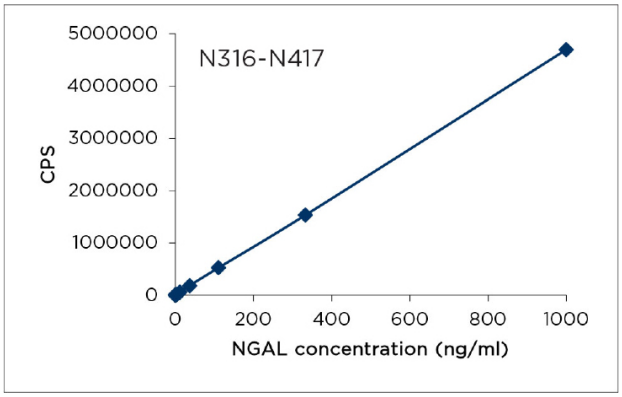


Figure 1. Calibration curve for MAb pair N316-N417.
Detection antibody was labelled with Eu3+. Recombinant NGAL (Cat.# 8-NGAL) was used as the antigen. The pair shows a wide linearity range.

Detection of NGAL in plasma samples

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

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

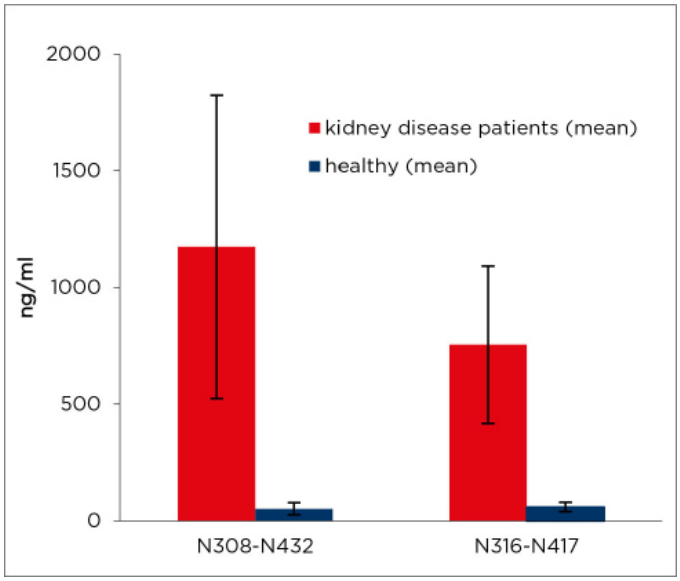


Figure 2. Mean concentrations of NGAL measured in patients diagnosed with a kidney disease and in apparently healthy individuals. *NGAL concentration in patients with a kidney disease was several fold higher than in healthy individuals when measured using two prototype assays: N308-N432 and N316-N417.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

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

Table 3. Spearman Rank Order Correlations for two prototype assays and Human NGAL ELISA (Kit 036CE) from Bioporto. *Marked correlations are significant at p <0,05000.*

Recombinant human NGAL

Advanced Immunochemical 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).

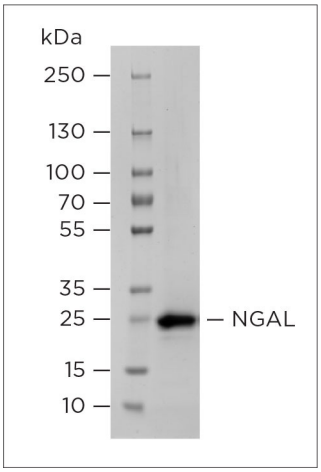


Figure 3. SDS-PAGE of recombinant NGAL under reducing conditions in a gradient gel (4-20%). *4 µg of purified protein was loaded on the gel.*

Parallel calibration curves for native and recombinant NGAL

The immunochemical properties of recombinant NGAL were compared 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.

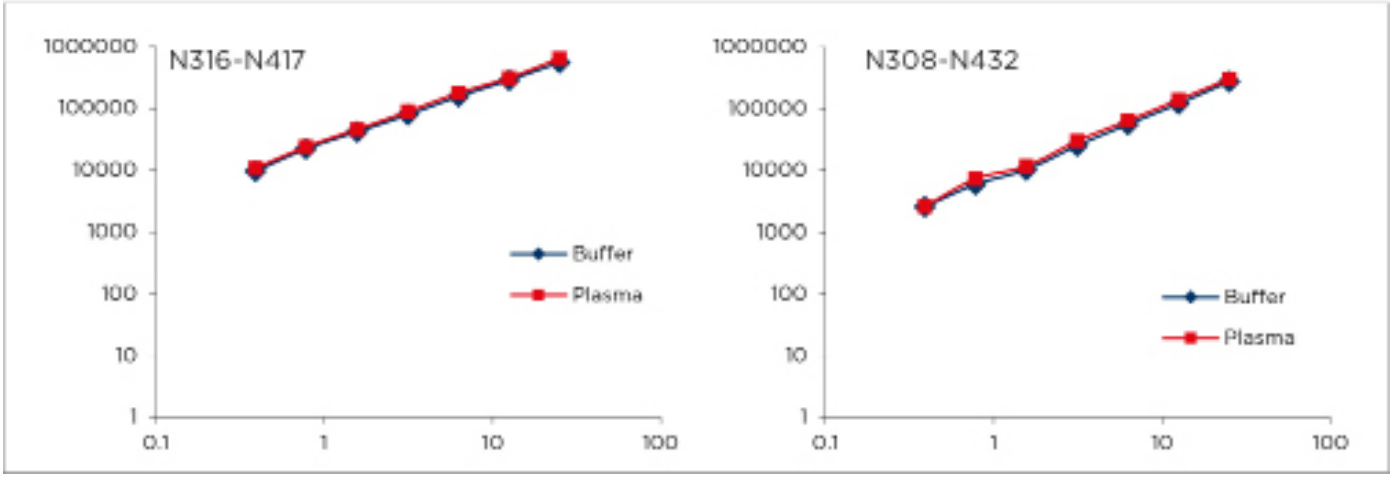


Figure 4. Titration curves for recombinant NGAL and native NGAL in human plasma using MAb pairs N316-N417 (A) and N308-N432 (B).

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAb	Subclass	Remarks
Neutrophil gelatinase-associated lipocalin (NGAL)	2-NGAL	N308	1gG	EIA, WB, recombinant rabbit antibody
		N316	1gG	EIA, WB, recombinant rabbit antibody
		N417	1gG1	*In vitro*, EIA, WB
		N422	1gG1	*In vitro*, EIA
		N432	1gG1	*In vitro*, EIA
		N457	1gG1	*In vitro*, EIA
		N461	1gG1	*In vitro*, EIA

ANTIGEN

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

References

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