

**Predictive Safety Testing Consortium Nephrotoxicity Working Group
Evaluation of BioPorto Diagnostics Monkey NGAL ELISA Kit (KIT 045)**

Introduction

This report summarizes analytical performance data for the Monkey NGAL ELISA kit (KIT 045) supplied by BioPorto Diagnostics to members of the Predictive Safety Testing Consortium (PSTC) Nephrotoxicity Working Group (NWG) for use in evaluating NGAL as a potential biomarker of renal injury in monkeys. Three sites performed technical validation of the NGAL assay for monkey urine samples, which included assessments of quantitation limits, precision, linearity, stability, and preliminary reference ranges. Each site performed validation testing independently and urine samples were sourced at individual sites. Results are summarized below.

Quantitation Limits

At one testing site, the standard curve was reproducible ($CV\% \leq 20\%$) across multiple plates and two reagent lots. An estimate of relative error of the mean back calculated concentrations compared to the nominal (true) value of each calibrator was within acceptable limits of $\pm 10\%$. Therefore, the lowest (10 pg/mL) and highest (1000 pg/ml) standards met acceptable precision and accuracy criteria to be the upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) for the NGAL assay, respectively. The ULOQ and LLOQ were confirmed at a second site.

Nominal Value (pg/mL)	STD-1	STD-2	STD-3	STD-4	STD-5	STD-6	STD-7
	1000	500	250	100	50	25	10
Plate 1	1001	499	254	103	48.2	23.7	10.5
Plate 2	1001	498	255	101	48.4	24.1	10.4
Plate 3	1001	498	255	101	48.3	24.3	10.3
Plate 4	1003	493	269	105	44.8	21.7	11.5
Plate 5	1001	497	259	102	47.7	23.2	10.7
Plate 6	1000	499	252	101	49.6	23.8	10.4
Plate 7	1000	500	250	102	48.2	25.4	10.0
Plate 8	1002	511	245	96.9	51.5	28.1	11.9
Plate 9	1001	506	248	97.5	51.0	27.3	11.2
Plate 10	1001	516	245	95.2	50.6	25.8	9.9
Mean	1001	501.7	253.2	100.46	48.83	24.74	10.68
SD	0.88	7.09	7.18	3.01	1.95	1.93	0.65
%CV	0.1	1.4	2.8	3.0	4.0	7.8	6.1
%Relative Error	0.1	0.3	1.3	0.5	-2.3	-1.0	6.8

Precision

Three sites reported intra- and inter-assay precision data using monkey urine samples with NGAL concentrations that spanned the standard curve range of the BioPorto NGAL assay. Results were within acceptable limits of CV% ≤ 20.

Intra-assay Precision				
Location	Urine ID	Mean (pg/ml)	#replicates	CV%
Site 1	Low 1	36.2	8	3.8
	Low 2	72.4	8	3.6
	Mid	652.9	8	8.6
	High	974.0	6	6.8
Site 2	Low	8.8	3	6.3
	Mid	126.8	3	4.4
	High	426.2	3	1.8
Site 3	Low	96.0	10	3.1
	Mid	226.9	10	5.3
	High	732.8	10	5.3

Inter-assay Precision				
Location	Urine ID	Mean (pg/ml)	#replicates	CV%
Site 1	Low 1	22.8	8	11.1
	Low 2	81.0	8	11.9
	Mid	643.4	8	12.4
	High	910.1	8	3.5
Site 2	Low	8.2	3	18.7
	Mid	129.8	3	4.3
	High	411.9	3	5.2
Site 3	Low	98.3	18	6.0
	High	407.7	18	8.3

Linearity of Dilution

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	% Recovery
Monkey Urine 1	1	294	-	-
	100	34.2	29.4	116
	200	15.7	14.7	107
	250	12.2	11.8	104
Monkey Urine 2	1	162	-	-
	100	16.6	16.2	102
	200	8.4	8.1	104
	250	6.8	6.5	104
Monkey Urine 3	500	616	-	-
	1000	269	308	87
	2000	136	154	88
	4000	67	7	87
	8000	35	3	90
	16000	16	1	85

Stability

Two sites confirmed long-term stability of urinary NGAL for 6 months at -80°C.

Two sites confirmed freeze/thaw stability of urinary NGAL up to 3X.

Ranges of NGAL in Monkey Urine

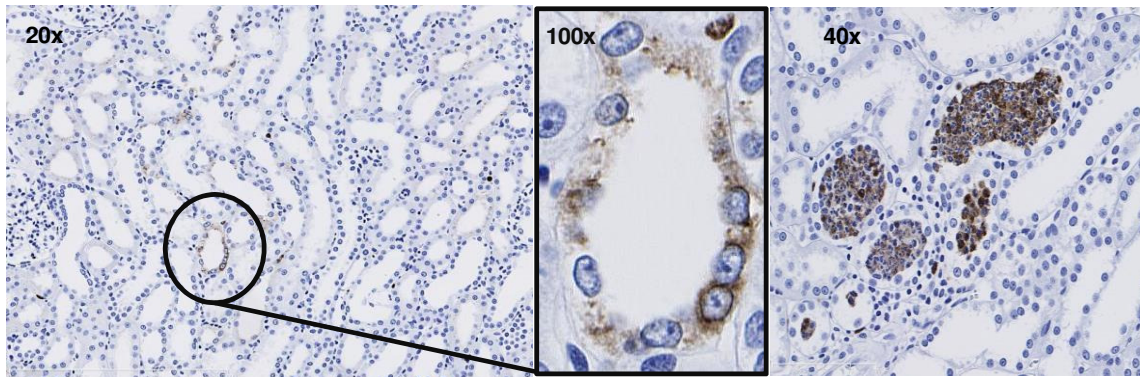
Urinary NGAL for untreated or naïve monkeys was collated across three sites and is summarized below.

NGAL was measured in the urine of monkeys following an overnight collection.

NGAL (ug/L)	Site 1		Site 2	Site 3	
	Male	Female	Male	Male	Female
Mean	10.4	37.2	4.1	11.4	57.1
SD	11.8	24.9	2.4	6.0	22.2
Min	2.5*	11.0	1.6	4.2	17.8
Max	60.5	87.0	11.5	21.1	80.8
N	44	9	12	8	12

*Value at or below LLOQ for 5 of 44 male monkeys

Immunohistochemistry (IHC) Staining of NHP Kidney



Immunohistochemistry staining for Compound-induced NGAL protein expression elevations in male Cynomolgus monkey using the BioPorto Diagnostics antibody (ABS063-56, clone 56) at 1:50. Mild multifocal staining was localized in apical surface of intact tubular epithelial cells in the cortex and medulla [left panel] and marked multifocal staining of tubular cast (granular and cellular) in the cortex [right panel].

Conclusion

The Predictive Safety Testing Consortium (PSTC) Nephrotoxicity Working Group (NWG) has determined that the BioPorto Diagnostics Monkey NGAL ELISA Kit (KIT 045) performance parameters (Precision, accuracy and dilutional linearity) were all within an acceptable range for the quantitation of urinary NGAL. Urinary NGAL concentrations were stable in urine samples that were subjected to three freeze/thaw cycles and for samples that were stored frozen at -80°C for 6 months. Furthermore, this assay kit is useful in the quantitation of urinary NGAL in nonclinical safety studies evaluating renal injury in monkeys.