

## Introduction

Interleukin-4 (IL-4) is a cytokine produced by various immune cells and has several important biological roles such as regulation of immune response, anti-inflammatory activity, tissue repair and remodeling and regulation of immunoglobulin isotype switching. Changes in the abundance of IL-4 in the blood or serum can serve as

biomarkers for various diseases and conditions. The NULISAqpcr IL-4 Assay only requires 20 µL of biological sample. The analytical performance of the NULISAqpcr IL-4 Assay has been carefully validated and the results are described below.

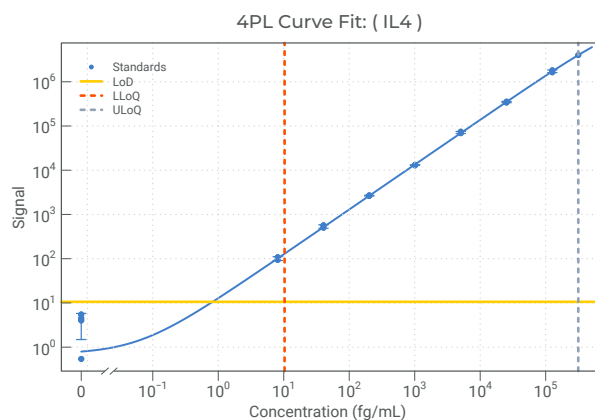
## NULISA™ Technology

NULISA is a sandwich immunoassay in which two target specific antibodies, conjugated with unique oligonucleotide tags, bind and form a complex with the specific protein in solution. The resulting immunocomplex is purified in sequential capture and release steps to remove background and unbound antibodies. Successful

formation of the immunocomplex brings the two oligonucleotide tags into proximity for a ligation reaction and the resulting product read out using quantitative PCR. The NULISAqpcr assay protocol is fully automated on the ARGO HT System which has an on-board qPCR reader and data is analyzed using the ARGO Command Center.

## Assay Performance

	Analytical	Functional
LOD	0.82 fg/mL	4.09 fg/mL
LLoQ	10.3 fg/mL	51.7 fg/mL
ULoQ	314,728 fg/mL	1,573,638 fg/mL
Dynamic Range	10.3–314,728 fg/mL	51.7–1,573,638 fg/mL



Assay performance was determined from seven independent runs using two different manufacturing lots of reagents on two different instrument systems.

The **LoD (Limit of Detection)** is the lowest concentration of the analyte that can be distinguished from the background signal of the assay and calculated as 2.5 standard deviations above the average of the blanks.

The **LLoQ (Lower Limit of Quantification)** is the lowest concentration of the analyte that can be reliably quantified by the assay and defined as the lowest concentration measurable by the assay with CV ≤ 25% and 75-125% recovery. The LLoQ was assessed from measurements of serially diluted calibrator material spiked into non-human matrix in triplicate in each of the 7 runs. LLoQ was calculated for each lot of reagents and the higher LLoQ value is reported.

The **ULoQ (Upper limit of Quantitation)** is the highest concentration of the analyte that can be reliably quantified by the assay and defined as the highest concentration measurable by the assay with CV ≤ 25% CV and 75-125% recovery. The ULoQ was assessed from measurements of serially diluted calibrator material spiked into non-human matrix in triplicate in each of the 7 runs. ULoQ was calculated for each lot of reagents and the lower ULoQ value is reported.

The **Dynamic range** is defined as the range of concentrations that an assay can reliably quantitate between the LLoQ to the ULoQ.

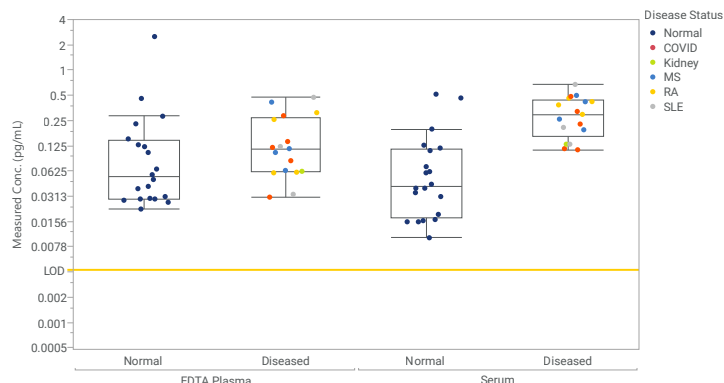
## Sample Controls

Internal and external controls are included in each run for data normalization and quality control purposes. Internal control is nucleic acid based and added to each well of the plate to monitor well to well variation and serves normalization purpose. External controls are High and

Low Quality Controls (QCs) included in the target kits, consisting of lyophilized plasma with predetermined IL4 concentrations. These QCs are treated as samples in the run to verify that the generated data consistently meet our stringent accuracy and precision criteria.

## Sample Detectability & Reference Ranges

Sample detectability and reference ranges were established with 37 EDTA plasma samples and 37 Serum samples. 20 normal and 17 diseased donors were tested for each matrix. The diseases represented consisted of COVID-19, Multiple Sclerosis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, and Kidney disease. 100% detectability was observed for each matrix.



	Plasma (pg/mL), n=37				Serum (pg/mL), n=37			
	Average	Median	Min	Max	Average	Median	Min	Max
<b>All</b>	0.194	0.083	0.022	2.509	0.198	0.127	0.010	0.674
<b>Normals</b>	0.221	0.053	0.022	2.509	0.100	0.041	0.010	0.516
<b>Diseased</b>	0.161	0.115	0.031	0.473	0.314	0.296	0.112	0.674
<b>Detectability</b>	100%				100%			

## Precision

Intra-assay precision or repeatability measures the variation in results obtained when a single sample is assayed multiple times within the same assay run. Inter-assay precision or reproducibility measures the variation in results obtained when the same sample

is assayed multiple times in different assay runs or on different days. Precision was determined by ANOVA analysis from a set of seven independent runs which included two reagent lots and two instruments.

Sample	Sample Category	Average Conc. (fg/mL)	Total %CV	Inter-assay %CV	Intra-assay %CV
PL-1	EDTA Plasma	156	16.6	0.0	11.3
PL-2		3,377	6.9	3.4	5.9
PL-3		15,820	5.8	0.0	4.4
C1	Assay Controls	52,062	6.7	4.4	5.1
C2		500	24.7	11.1	9.4
S1	Calibrator Dilution Series in Sample Dilution Buffer	1,566,589	12.2	7.7	4.2
S2		1,125,953	20.3	0.0	20.3
S3		998,242	5.9	0.8	4.7
S4		146,933	12.0	0.0	12.0
S5		16,308	6.4	0.0	5.4
S6		3,344	5.2	0.0	5.2
S7		826	8.5	5.9	6.1
S8		180	10.2	5.9	8.3
S9		89	15.3	4.1	12.7

## Parallelism

Parallelism confirms that the binding characteristic of the endogenous analyte to the antibodies is the same as for the calibrator. Each individual sample undergoes a serial dilution. Subsequently, the dilution series for each sample is assessed for recovery to ensure a consistent response across all samples.

Parallelism was assessed with 5 EDTA plasma samples and 5 serum samples serially diluted 2x with sample dilution buffer. At least 80% of the samples tested displayed parallelism with an average of 95% recovery in EDTA samples and 96% in serum samples across all dilution levels above the assay LLOQ.

## Spike Recovery

Spike recovery determines if the concentration–response relationship is similar in the calibration curve/buffer and the samples and is a way to look at matrix effect. A known amount of analyte is spiked into the natural test sample matrix and its response is measured (recovery) in the assay by comparison to an identical amount spiked into the standard diluent.

Spike recovery was determined with 5 EDTA plasma samples and 5 serum samples which were spiked to three concentrations and compared with spike into sample diluent.

$$\% \text{ Recovery} = \frac{\text{Meas. Conc Spiked Donor Sample} - \text{Meas. Conc Unspiked Donor Sample}}{\text{Meas. Conc Spiked SD buffer}} \times 100$$

Targeted Spike Conc (pg/mL)	EDTA Plasma (n = 5)			Serum (n = 5)		
	Avg Recovery	Recovery Range		Avg Recovery	Recovery Range	
2	75%	47	- 99%	101%	92	- 108%
50	87%	62	- 102%	88%	85	- 92%
250	99%	74	- 119%	97%	93	- 105%
All levels	87%	47	- 119%	95%	85	- 108%

## Dilution Linearity

Dilution linearity demonstrates that a sample with an analyte concentration above the ULOQ can be diluted to concentration within the assay dynamic range and give a reliable result. Normal human EDTA plasma and

serum were spiked with recombinant IL4 to above ULOQ concentrations and then serially diluted with sample diluent 5x. Percent recovery was normalized to the dilution adjusted, neat concentration.

	Plasma Donor 1	Plasma Donor 2	Plasma Donor 3	Serum Donor 4	Serum Donor 5	Serum Donor 6
D1 Concentration (pg/mL)	149.01	161.02	153.39	167.28	144.82	168
D2 Recovery (1/5 dilution)	93%	91%	97%	99%	107%	92%
D3 Recovery (1/25 dilution)	105%	85%	111%	104%	110%	97%
D4 Recovery (1/125 dilution)	107%	99%	116%	108%	124%	109%
D5 Recovery (1/625 dilution)	109%	97%	98%	101%	121%	97%
D6 Recovery (1/3125 dilution)	126%	97%	122%	112%	129%	112%
Average	104%			108%		
Min Recovery	85%			92%		
Max Recovery	126%			129%		

## Specificity

To ensure that every NULISAqpcr immunoassay accurately detects only the target molecule that it is designed to detect we test the cross reactivity with related proteins and potential interference from chaperones and potential binding partners, for each target.

Specificity and cross-reactivity were examined by measuring samples spiked with IL-4 and with potential cross-reactants added at 10x and 100x the molar

concentration of IL-4 and recovery of IL-4 was assessed by comparing to the IL-4 neat control. IL-4 samples spiked with potential cross-reactants showed acceptable recovery well within 70-130%.

Additionally, cross-reactants were tested without the presence of IL-4 to check for non-specific signal. Non-specific binding was observed to be  $\leq 0.1\%$ .

Cross-Reactant	Percent Recovery	
	Cross-Reactant molar concentration fold vs IL-4	
	10x	100x
IL-2RA	106.9%	117.8%
IL-4R	106.7%	111.9%
IL-6	106.8%	115.3%
IL-10	98.4%	101.1%
IL-13	100.9%	105.8%
IL-13RA1	100.8%	104.0%
IL-13RA2	103.0%	116.9%
RRAGA	99.2%	103.8%
RRAGB	104.3%	108.7%

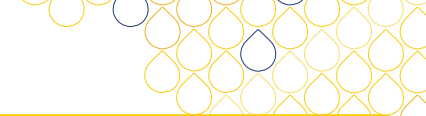
Cross-Reactant	%Non-Specificity vs IL-4 spike
	Cross-Reactant amount
	10x
IL-2RA	0.055%
IL-4R	0.045%
IL-6	0.097%
IL-10	0.056%
IL-13	0.032%
IL-13RA1	0.106%
IL-13RA2	0.079%
RRAGA	0.073%
RRAGB	0.048%

## Interference

Interference screening was performed in the presence of three concentration of IL4. No interference was observed for the substances screened in the table.

Substance	Concentration
Bilirubin	342 $\mu$ M
Biotin	30 ng/mL
Fibrinogen	400 mg/dL
HAMA	100 ng/mL
Hemoglobin	2 g/L
Rheumatoid Factor	150 IU/mL
Triglycerides	1000 mg/dL
RRAGA	99.2%
RRAGB	104.3%

# NULISAqpcr™ IL-4 Assay



## Sample stability and handling recommendations

Sample stability testing has shown that samples can be stored up to at least 4 hours at room temperature, up to at least 4 days in 4°C, and can withstand up to at least 3 freeze-thaw cycles.

Recovery				
Storage Condition	Time Point	Plasma IL4, 0.71 pg/mL	Plasma IL4, 2.92 pg/mL	Plasma IL4, 11.97 pg/mL
RT	2 hours	104%	101%	122%
	4 hours	82%	90%	91%
4° C	24 hours	99%	92%	91%
	48 hours	93%	91%	98%
	96 hours	83%	100%	106%
Freeze-Thaw	1 cycle	93%	101%	96%
	3 cycles	85%	93%	98%

## Ordering Information

### NULISAqpcr Assays

Product Name	Plate Format	Sample Type	Catalog Number
NULISAqpcr IL-4 Assay	96	plasma/serum	800107

### Consumables & Buffers

Product Name	Qty	Catalog Number
NULISA Wash Buffer (3L)	3L	801035

### Instrument

Product Name	Qty	Catalog Number
Alamar ARGO HT System	1	800101

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