# NULISAqpcr™ IL-4 Assay

DATA SHEET

## Introduction

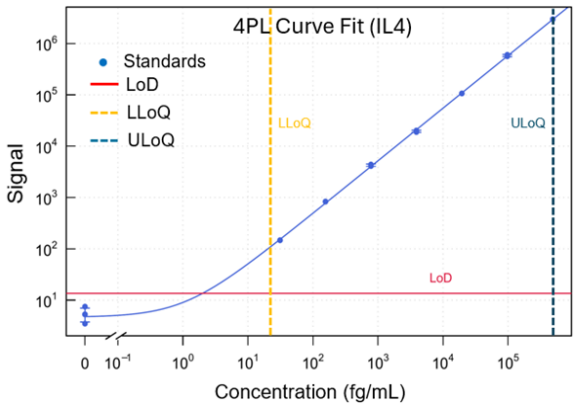
Interleukin-4 (IL-4) is a cytokine produced by various immune cells and has several important biological roles such as regulation of immune response, anti-inflammatory activity, tissue repair and remodeling, and regulation of immunoglobulin isotype switching. Changes in the abundance of IL-4 in the blood or serum can serve as a biomarker for various diseases and conditions. The NULISAqpcr IL-4 Assay only requires 20 µL of biological sample per reaction. 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 oligonucleotides 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 | 0.26 fg/mL | 1.31 fg/mL |
| LLoQ | 4.4 fg/mL | 22.0 fg/mL |
| ULoQ | 97,476 fg/mL | 487,380 fg/mL |
| Dynamic Range | 4.4–97,476 fg/mL | 22.0–487,380 fg/mL |




Assay performance was determined from eighteen independent runs using one manufacturing lot of reagents on five 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 **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 **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. The internal control is a nucleic acid based oligo 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 IL-4 concentrations. These QCs are treated as samples in the run to verify that the generated data consistently meet our stringent accuracy and precision criteria.

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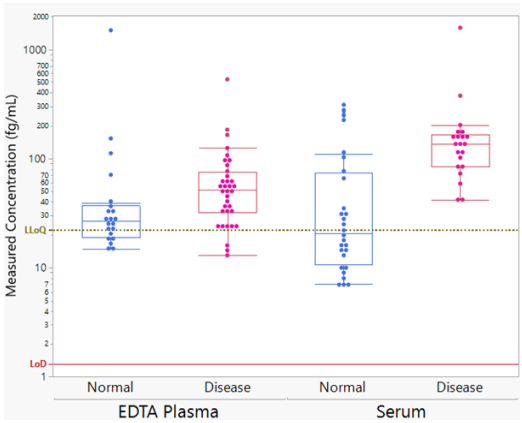
## Sample Detectability & Reference Ranges

Sample detectability and reference ranges were established with 58 EDTA plasma samples (22 normal and 36 diseased) and 48 Serum samples (28 normal and 20 diseased). The diseased samples consisted of COVID-19, Multiple Sclerosis (MS), Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Myeloma, Cancer, Chronic lymphocytic leukemia (CLL), and kidney disease. 100% detectability was observed for each matrix.




| | Plasma (fg/mL), n=58 | | | | Serum (fg/mL), n=48 | | | |
|---|---|---|---|---|---|---|---|---|
| | Average | Median | Min | Max | Average | Median | Min | Max |
| **All** | 84 | 35 | 13 | 1500 | 122 | 73 | 7 | 1583 |
| **Normals** | 101 | 27 | 15 | 1500 | 117 | 21 | 7 | 311 |
| **Diseased** | 61 | 52 | 13 | 533 | 152 | 136 | 42 | 1583 |
| **Detectability** | 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 four independent runs which included one reagent lot and two instruments.

| Sample | Sample Category | Average Conc. (fg/mL) | Total %CV | Inter-assay %CV | Intra-assay %CV |
|---|---|---|---|---|---|
| PL-1 | EDTA Plasma | 920 | 10.46 | 7.66 | 7.13 |
| PL-2 | | 15,220 | 7.15 | 1.57 | 6.98 |
| PL-3 | | 111,870 | 4.55 | 1.91 | 4.14 |
| C1 | Assay Controls | 38,840 | 9.87 | 8.26 | 5.4 |
| C2 | | 730 | 14.91 | 13.28 | 6.78 |

# NULISAqpcr™ IL-4 Assay

## 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 3 EDTA plasma samples and 6 serum samples serially diluted 2x with sample dilution buffer. 88.9% of the samples tested displayed parallelism with an average of 105% recovery in EDTA samples and 102% in serum samples across all dilution levels above the assay LLoQ.

## Spike Recovery

Examining the matrix effect throug spike recovery determines if the concentration–response relationship is similar between the calibration curve/buffer and samples. A known amount of analyte is spiked into the native test sample matrix and its response is measured (recovery) in the assay by comparison to an identical amount spiked into the standard diluent.

5 EDTA plasma samples which were spiked with three analyte concentrations and subsequently compared with spike into sample diluent. At least 80% of the samples showed a recovery within 70% ~ 130%.

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

| Targeted Spike Conc (fg/mL) | Avg Recovery | Recovery Range | | |
|---|---|---|---|---|
| 310 | 90% | 71 | - | 101% |
| 50,000 | 86% | 82 | - | 88% |
| 350,000 | 103% | 98 | - | 112% |

## Dilution Linearity

Dilution linearity demonstrates that a sample with an analyte concentration above the ULoQ can be diluted to a concentration within the assay dynamic range and give a reliable result. Four human EDTA plasma and one serum sample were spiked with recombinant IL-4 and then 2x serially diluted with sample diluent for up to 128x. Percent recovery was normalized to adjust for the dilution factor.

| | Plasma Donor 1 | Plasma Donor 2 | Serum Donor 3 | Plasma Donor 4 | Plasma Donor 5 |
|---|---|---|---|---|---|
| **Starting Concentration (fg/mL)** | 297,100 | 304,600 | 316,300 | 276,300 | 304,800 |
| **Average Recovery** | 104% | 111% | 96% | 104% | 104% |
| **Min Recovery** | 96% | 102% | 87% | 96% | 91% |
| **Max Recovery** | 111% | 118% | 103% | 111% | 116% |

# NULISAqpcr™ IL-4 Assay

## 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 analyte and with a panel of human analytes (specificity panel) 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 the specificity panel showed acceptable recovery well within 70-130%.

Additionally, the specificity panel was tested without the presence of IL-4 to check for non-specific signal. Non-specific binding was observed to be ≤ 0.1%.

| | Percent Recovery<br>Specificity panel molar concentration fold vs IL-4 | |
|---|---|---|
| Cross-Reactant | 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% |

| | %Non-Specificity vs IL-4 spike<br>Specific panel amount |
|---|---|
| Cross-Reactant | 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 concentrations of IL-4. No interference was observed for the screened substances listed in the table.

| Substance | Concentration |
|---|---|
| Bilirubin | 342 µM |
| 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 |

# 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 IL-4, 0.71 pg/mL | Plasma IL-4, 2.92 pg/mL | Plasma IL-4, 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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