

## Introduction

Inflammation and disruptions in immune response represent key mechanisms in the development and treatment of many diseases. Detection and measurement of key immune related proteins provides critical information in the discovery and screening for biomarkers associated with disease progression or therapeutic response. The NULISAseq Inflammation Panel AQ measures the absolute concentrations of 156 cytokines,

chemokines and inflammation-related human proteins present in biofluids such as plasma and serum. In addition, the panel measures the relative abundance of these proteins as well as 94 additional proteins, covering the complete profile of the existing Inflammation RQ product. The analytical performance of the product has been carefully validated and the results are presented below.

## NULISA™ Technology

NULISAseq is a sandwich immunoassay in which 2 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 amplification. The resulting unique oligonucleotide reporters containing both target and sample information are then pooled to a library for analysis on an NGS sequencer. The assay protocol

is fully automated on the ARGO HT System and data is analyzed using the NULISA Analysis Software. More details on the assay protocol can be found [here](#).



## Data Normalization and Quantification Methods

For relative quantification (RQ) NULISA Protein Quantification (NPQ) units are log<sub>2</sub>-scale values used to quantify relative protein abundances. NPQ is derived from the raw sequencing reads using the following normalization and transformation steps. First, to control for intra-plate well-to-well variation, the raw sequencing read count for each analyte for a given sample well is divided by that well's internal control (IC) raw count. Second, to control for plate-to-plate variation, the IC-normalized values for each analyte are divided by the analyte-specific median IC-normalized counts from the 3 calibrator (CAL) samples on the plate. Data is then rescaled and log<sub>2</sub>-transformed to obtain the data in NPQ, which are approximately normally distributed values amenable to downstream statistical analysis.

For absolute quantification (AQ), data is normalized using the IC and CAL samples as described for RQ. Next, a run-specific 4PL curve is generated for each target by combining information from pre-determined master curve parameters, known CAL concentrations, and the CAL normalized counts using Alamar's AQ algorithm. These curves are then used to generate absolute concentrations in pg/mL and aM for the AQ targets in each sample.



## Sensitivity & Quantifiability

The limit of detection (LOD), or the lowest concentration of the analyte that can be distinguished from the background signal of the assay, is calculated for each target as the mean plus three standard deviations of the 4 negative control (NC) wells' normalized reads. A maximum of one outlier NC value may be omitted.

For AQ targets, LOD was determined in absolute concentration using a set of 3 additional runs. The mean plus three standard deviations of the 4 NC wells' normalized reads were converted to pg/mL using Alamar's proprietary algorithm (Table 2).

## Precision

For AQ targets, coefficient of variation (CV) was assessed using a set of 8 runs including 14 plasma samples with 3 technical replicates each. The plasma samples were spiked with different concentrations of recombinant antigen pools (13-point and 2-fold serial dilutions) and a sample diluent buffer. Data were quantified in absolute concentration using Alamar's AQ algorithm. Values outside of the dynamic range were excluded, and variance component models were then fit for each sample and target and included the factors reagent lot, instrument, inter-plate, and intra-plate. For each target, the estimated CV for each component was averaged across the 14 samples (Table 2). Table 1 shows across-target medians for each CV component.

Table 1: Across-target Median AQ Coefficients of Variation

| CV component      | Median CV (%), Plasma |
|-------------------|-----------------------|
| Intra-plate       | 5.96                  |
| Inter-plate       | 3.07                  |
| Inter-Instrument  | 0.95                  |
| Inter-Reagent Lot | 1.75                  |
| Total             | 8.35                  |

## Dilutional Linearity

Dilutional linearity was assessed for each target using 13-point 2-fold dilutions of plasma samples with spiked-in recombinant antigen. Samples were quantified using Alamar's AQ algorithm, and values outside dynamic range were excluded. Recovery error was calculated by multiplying the measured concentration of the diluted samples by their dilution factor and dividing the result

by the measured concentration of the first dilution point below ULoQ times its own dilution factor. A <30% recovery error for at least 75% of samples within the dynamic range was required for each AQ target. Table 3 reports the average recovery error for all samples in the dynamic range for each target.

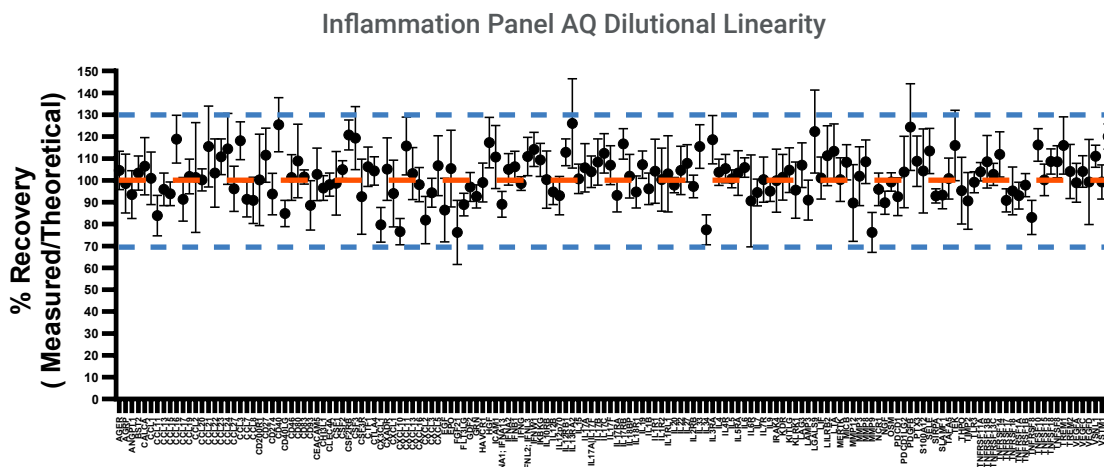


Table 2: Performance Validation Data by Target

| Target     | UniProt ID | Protein Name                                              | Quantifiability (%) |              | AQ Precision (CV%) |       |      |       | LOD (pg/mL) | LLOQ (pg/mL) | ULOQ (pg/mL) | log10 Range | Dilution Linearity (%) |
|------------|------------|-----------------------------------------------------------|---------------------|--------------|--------------------|-------|------|-------|-------------|--------------|--------------|-------------|------------------------|
| AQ Targets |            |                                                           | Plasma              |              | Plasma             |       |      |       |             |              |              |             |                        |
|            |            |                                                           | Healthy n=57        | Disease n=62 | Intra              | Inter | Lot  | Total |             |              |              |             |                        |
| AGER       | Q15109     | Advanced glycosylation end product-specific receptor      | 100                 | 100          | 4.5                | 3.7   | 1.3  | 7.3   | 0.679       | 15.915       | 69821        | 3.6         | 104                    |
| AGRP       | O00253     | Agouti-related protein                                    | 98                  | 100          | 5.1                | 3.6   | 0.7  | 6.7   | 0.217       | 4.471        | 250375       | 4.7         | 98                     |
| ANGPT1     | Q15389     | Angiopoietin-1                                            | 100                 | 100          | 5.0                | 2.3   | 2.2  | 6.8   | 43.715      | 70.724       | 1301018      | 4.3         | 94                     |
| BST2       | Q10589     | Bone marrow stromal antigen 2                             | 100                 | 100          | 6.3                | 3.3   | 1.7  | 7.8   | 0.608       | 5.946        | 522812       | 4.9         | 103                    |
| CALCA      | P01258     | Calcitonin [Cleaved into: Calcitonin; Katalcalcin         | 100                 | 100          | 6.0                | 2.0   | 1.0  | 7.2   | 0.239       | 1.394        | 276417       | 5.3         | 106                    |
| CCL1       | P22362     | C-C motif chemokine 1                                     | 96                  | 100          | 4.0                | 2.0   | 4.1  | 7.5   | 0.006       | 0.453        | 105          | 2.4         | 101                    |
| CCL11      | P51671     | Eotaxin                                                   | 98                  | 100          | 5.2                | 2.9   | 0.2  | 6.3   | 1.300       | 7.932        | 1237052      | 5.2         | 84                     |
| CCL13      | Q99616     | C-C motif chemokine 13                                    | 100                 | 100          | 5.8                | 3.1   | 5.5  | 9.7   | 0.592       | 1.354        | 43854        | 4.5         | 96                     |
| CCL15      | Q16663     | C-C motif chemokine 15                                    | 100                 | 100          | 5.9                | 2.6   | 7.8  | 12.0  | 141.915     | 673.672      | 193563       | 2.5         | 94                     |
| CCL16      | O15467     | C-C motif chemokine 16                                    | 100                 | 100          | 8.1                | 2.0   | 0.9  | 9.1   | n/a         | 329.066      | 396353       | 3.1         | 119                    |
| CCL17      | Q92583     | C-C motif chemokine 17                                    | 84                  | 95           | 4.2                | 2.8   | 5.6  | 8.5   | 13.695      | 52.662       | 30325        | 2.8         | 91                     |
| CCL19      | Q99731     | C-C motif chemokine 19                                    | 100                 | 98           | 5.5                | 6.3   | 0.8  | 10.4  | 1.284       | 5.836        | 47338        | 3.9         | 102                    |
| CCL2       | P13500     | C-C motif chemokine 2                                     | 98                  | 100          | 5.6                | 4.9   | 8.1  | 12.8  | 2.817       | 15.340       | 23367        | 3.2         | 101                    |
| CCL20      | P78556     | C-C motif chemokine 20                                    | 32                  | 40           | 8.0                | 5.1   | 2.4  | 11.1  | 2.894       | 6.852        | 22395        | 3.5         | 100                    |
| CCL21      | O00585     | C-C motif chemokine 21                                    | 100                 | 100          | 4.7                | 2.8   | 1.4  | 6.5   | 2.452       | 32.885       | 33837        | 3.0         | 116                    |
| CCL22      | O00626     | C-C motif chemokine 22                                    | 100                 | 100          | 6.5                | 3.8   | 5.0  | 11.1  | 7.879       | 37.458       | 74549        | 3.3         | 103                    |
| CCL23      | P55773     | C-C motif chemokine 23                                    | 98                  | 100          | 7.5                | 3.5   | 4.0  | 10.7  | 0.422       | 37.500       | 214123       | 3.8         | 111                    |
| CCL24      | O00175     | C-C motif chemokine 24                                    | 100                 | 100          | 5.2                | 3.3   | 2.1  | 7.4   | 158.818     | 235.465      | 203475       | 2.9         | 114                    |
| CCL27      | Q9Y4X3     | C-C motif chemokine 27                                    | 100                 | 100          | 6.1                | 3.5   | 0.6  | 7.4   | 1.375       | 4.270        | 81010        | 4.3         | 96                     |
| CCL3       | P10147     | C-C motif chemokine 3                                     | 98                  | 100          | 9.8                | 5.2   | 9.0  | 16.7  | 0.171       | 0.443        | 3538         | 3.9         | 118                    |
| CCL7       | P80098     | C-C motif chemokine 7                                     | 98                  | 100          | 4.5                | 2.8   | 0.4  | 5.7   | 0.017       | 0.036        | 236          | 3.8         | 91                     |
| CCL8       | P80075     | C-C motif chemokine 8                                     | 88                  | 97           | 5.7                | 4.2   | 23.9 | 27.2  | 0.747       | 5.157        | 2890         | 2.7         | 91                     |
| CD200R1    | Q8TD46     | Cell surface glycoprotein CD200 receptor 1                | 98                  | 100          | 6.9                | 4.1   | 0.9  | 8.9   | n/a         | 117.730      | 1000446      | 3.9         | 100                    |
| CD27       | P26842     | CD27 antigen                                              | 98                  | 100          | 7.5                | 4.2   | 0.7  | 9.0   | 36.232      | 409.530      | 2159850      | 3.7         | 112                    |
| CD274      | Q9NZQ7     | Programmed cell death 1 ligand 1, PD-L1                   | 98                  | 100          | 6.4                | 3.2   | 2.4  | 8.2   | 0.584       | 2.256        | 145637       | 4.8         | 94                     |
| CD40       | P25942     | CD40 molecule                                             | 100                 | 100          | 7.6                | 3.4   | 1.7  | 9.9   | 1.727       | 11.034       | 44290        | 3.6         | 125                    |
| CD40LG     | P29965     | CD40 ligand                                               | 89                  | 100          | 4.2                | 3.6   | 2.3  | 6.4   | 0.099       | 2.028        | 355320       | 5.2         | 85                     |
| CD46       | P15529     | Membrane cofactor protein                                 | 98                  | 100          | 6.0                | 1.5   | 1.6  | 7.3   | 515.791     | 2714.223     | 1797419      | 2.8         | 101                    |
| CD80       | P33681     | T-lymphocyte activation antigen CD80                      | 100                 | 100          | 6.0                | 2.2   | 2.1  | 7.5   | 0.370       | 1.558        | 76088        | 4.7         | 109                    |
| CD83       | Q01151     | CD83 antigen                                              | 100                 | 100          | 4.2                | 6.4   | 0.5  | 9.1   | 0.041       | 0.067        | 17406        | 5.4         | 101                    |
| CD93       | Q9NPY3     | Complement component C1q receptor                         | 98                  | 100          | 6.9                | 2.0   | 0.5  | 8.0   | 201.466     | 3648.162     | 3590956      | 3.0         | 89                     |
| CEA-CAM5   | P06731     | Carcinoembryonic antigen related cell adhesion molecule 5 | 100                 | 100          | 6.8                | 1.9   | 0.9  | 7.7   | 35.790      | 46.248       | 1958077      | 4.6         | 103                    |
| CHI3L1     | P36222     | Chitinase-3-like protein 1                                | 100                 | 100          | 7.8                | 2.1   | 2.2  | 9.2   | 237.854     | 1271.827     | 2127192      | 3.2         | 96                     |
| CLEC4A     | Q9UMR7     | C-type lectin domain family 4 member A                    | 100                 | 100          | 4.6                | 2.8   | 1.8  | 6.6   | 0.426       | 2.136        | 114283       | 4.7         | 98                     |
| CSF1       | P09603     | Macrophage colony-stimulating factor 1                    | 98                  | 100          | 7.5                | 5.8   | 8.9  | 15.3  | 6.722       | 24.314       | 95036        | 3.6         | 99                     |
| CSF2       | P04141     | Granulocyte-macrophage colony-stimulating factor          | 98                  | 100          | 4.1                | 3.6   | 0.5  | 6.3   | 0.002       | 0.012        | 713          | 4.8         | 105                    |
| CSF2RB     | P32927     | Cytokine receptor common subunit beta                     | 100                 | 100          | 3.8                | 1.9   | 1.7  | 5.2   | 0.212       | 1.496        | 229764       | 5.2         | 121                    |
| CSF3       | P09919     | Granulocyte colony-stimulating factor, G-CSF              | 100                 | 95           | 4.6                | 2.6   | 3.4  | 7.5   | 0.376       | 2.313        | 16021        | 3.8         | 119                    |
| CSF3R      | Q99062     | Granulocyte colony-stimulating factor receptor            | 100                 | 100          | 5.3                | 2.8   | 0.1  | 6.6   | 0.913       | 11.499       | 763516       | 4.8         | 92                     |
| CTF1       | Q16619     | Cardiotrophin-1                                           | 100                 | 100          | 6.1                | 2.1   | 3.6  | 8.9   | 0.071       | 0.221        | 59481        | 5.4         | 106                    |
| CTLA4      | P16410     | Cytotoxic T-lymphocyte protein 4                          | 100                 | 100          | 4.1                | 3.2   | 0.7  | 5.6   | 0.009       | 0.009        | 3599         | 5.6         | 104                    |
| CX3CL1     | P78423     | Fractalkine                                               | 100                 | 100          | 5.6                | 3.8   | 3.0  | 8.1   | 3.455       | 13.955       | 603348       | 4.6         | 80                     |
| CXADR      | P78310     | Coxsackievirus and adenovirus receptor                    | 100                 | 100          | 5.7                | 3.2   | 0.2  | 7.2   | 0.804       | 0.896        | 38329        | 4.6         | 105                    |

Table 2: Performance Validation Data by Target (cont.)

| Target         | UniProt ID     | Protein Name                                             | Quantifiability (%) |              | AQ Precision (CV%) |       |      |       | LOD (pg/mL) | LLOQ (pg/mL) | ULOQ (pg/mL) | log10 Range | Dilution Linearity (%) |
|----------------|----------------|----------------------------------------------------------|---------------------|--------------|--------------------|-------|------|-------|-------------|--------------|--------------|-------------|------------------------|
| AQ Targets     |                |                                                          | Plasma              |              | Plasma             |       |      |       |             |              |              |             |                        |
|                |                |                                                          | Healthy n=57        | Disease n=62 | Intra              | Inter | Lot  | Total |             |              |              |             |                        |
| CXCL1          | P09341         | Growth-regulated alpha protein                           | 82                  | 100          | 7.7                | 4.3   | 2.3  | 10.5  | 7.376       | 12.282       | 279369       | 4.4         | 94                     |
| CXCL10         | P02778         | C-X-C motif chemokine 10                                 | 98                  | 100          | 6.7                | 3.0   | 0.6  | 8.4   | 1.547       | 8.853        | 47757        | 3.7         | 77                     |
| CXCL11         | O14625         | C-X-C motif chemokine 11                                 | 91                  | 98           | 4.9                | 4.7   | 15.1 | 18.7  | 0.877       | 4.624        | 3317         | 2.9         | 116                    |
| CXCL13         | O43927         | C-X-C motif chemokine 13                                 | 7                   | 31           | 9.9                | 4.2   | 0.0  | 12.3  | n/a         | 51.435       | 75597        | 3.2         | 103                    |
| CXCL16         | Q9H2A7         | C-X-C motif chemokine 16                                 | 100                 | 100          | 6.1                | 2.4   | 4.2  | 9.2   | 7.978       | 97.116       | 284781       | 3.5         | 98                     |
| CXCL2          | P19875         | C-X-C motif chemokine ligand 2                           | 61                  | 65           | 6.2                | 3.9   | 2.2  | 8.6   | 3.273       | 57.561       | 39403        | 2.8         | 82                     |
| CXCL3          | P19876         | C-X-C motif chemokine ligand 3                           | 100                 | 98           | 7.3                | 1.2   | 5.1  | 10.1  | 3.206       | 11.142       | 45369        | 3.6         | 94                     |
| CXCL5          | P42830         | C-X-C motif chemokine 5                                  | 100                 | 100          | 7.6                | 1.0   | 0.5  | 8.8   | 0.151       | 4.234        | 241579       | 4.8         | 107                    |
| EGF            | P01133         | Pro-epidermal growth factor                              | 100                 | 100          | 6.3                | 3.7   | 0.4  | 8.6   | 0.212       | 0.739        | 29609        | 4.6         | 86                     |
| EPO            | P01588         | Erythropoietin                                           | 98                  | 100          | 6.5                | 3.6   | 1.4  | 8.9   | 1.817       | 2.942        | 38639        | 4.1         | 105                    |
| FGF21          | Q9NSA1         | Fibroblast growth factor 21                              | 98                  | 98           | 5.6                | 3.5   | 2.0  | 8.1   | 58.556      | 117.120      | 1819547      | 4.2         | 76                     |
| FLT3LG         | P49771         | Fms-related tyrosine kinase 3 ligand                     | 98                  | 100          | 7.0                | 11.0  | 12.7 | 18.8  | 0.624       | 5.644        | 5131         | 3.0         | 89                     |
| GDF2           | Q9UK05         | Growth/differentiation factor 2                          | 98                  | 100          | 7.9                | 2.8   | 4.4  | 12.3  | 7.644       | 16.526       | 15054        | 3.0         | 97                     |
| GRN            | P28799         | Progranulin                                              | 98                  | 100          | 9.6                | 6.1   | 4.8  | 14.6  | 549.286     | 1708.600     | 4052298      | 3.4         | 93                     |
| HAVCR1         | Q96D42         | Hepatitis A virus cellular receptor 1                    | 100                 | 100          | 5.3                | 2.4   | 0.5  | 6.4   | n/a         | 1.146        | 194173       | 5.2         | 99                     |
| HGF            | P14210         | Hepatocyte growth factor                                 | 100                 | 100          | 9.0                | 3.9   | 1.1  | 12.0  | 0.373       | 1.163        | 25452        | 4.3         | 117                    |
| ICAM1          | P05362         | Intercellular adhesion molecule 1                        | 100                 | 100          | 7.4                | 1.7   | 2.1  | 9.0   | 519.723     | 2386.600     | 970313       | 2.6         | 111                    |
| IFNA1; IFNA13  | P01562; P01562 | Interferon alpha-1; Interferon alpha-13                  | 93                  | 95           | 4.3                | 3.5   | 0.6  | 6.6   | 0.001       | 0.005        | 21053        | 6.7         | 89                     |
| IFNA2          | P01563         | Interferon alpha-2                                       | 19                  | 31           | 5.6                | 3.2   | 2.0  | 7.6   | 0.004       | 0.015        | 2264         | 5.2         | 105                    |
| IFNB1          | P01574         | Interferon beta                                          | 54                  | 56           | 7.4                | 2.8   | 13.5 | 17.9  | 0.002       | 0.018        | 1050         | 4.8         | 106                    |
| IFNG           | P01579         | Interferon gamma                                         | 100                 | 100          | 3.6                | 0.9   | 1.4  | 5.1   | 0.005       | 0.021        | 23445        | 6.1         | 98                     |
| IFNL2; IFNL3   | Q8IZJ0; Q8IZI9 | Interferon lambda-2; Interferon lambda-3                 | 61                  | 84           | 6.3                | 5.6   | 0.8  | 9.1   | 0.028       | 0.081        | 6901         | 4.9         | 111                    |
| IFNW1          | P05000         | Interferon omega-1                                       | 5                   | 16           | 5.6                | 2.1   | 0.7  | 6.6   | 0.001       | 0.016        | 892          | 4.8         | 114                    |
| IKBKG          | Q9Y6K9         | Inhibitor of nuclear factor kappa B kinase subunit gamma | 100                 | 100          | 4.9                | 2.0   | 1.8  | 6.4   | 5.946       | 15.363       | 2257463      | 5.2         | 109                    |
| IL10RB         | Q08334         | Interleukin-10 receptor subunit beta                     | 100                 | 100          | 5.9                | 3.6   | 1.0  | 7.7   | 3.364       | 12.522       | 1340541      | 5.0         | 100                    |
| IL12B          | P29460         | Interleukin-12 subunit beta                              | 100                 | 100          | 4.9                | 1.6   | 0.3  | 5.9   | 0.366       | 0.615        | 151026       | 5.4         | 95                     |
| IL12p70        | P29459; P29460 | Interleukin-12 subunit beta Interleukin-12 subunit alpha | 96                  | 98           | 6.0                | 3.5   | 0.2  | 7.5   | 0.003       | 0.027        | 3682         | 5.1         | 93                     |
| IL12RB1        | P42701         | Interleukin-12 receptor subunit beta-1                   | 96                  | 100          | 5.1                | 1.6   | 1.2  | 6.4   | 4.305       | 5.696        | 258420       | 4.7         | 113                    |
| IL13RA2        | Q14627         | Interleukin 13 receptor subunit alpha 2                  | 98                  | 100          | 5.9                | 4.6   | 2.7  | 9.9   | 1.545       | 7.591        | 375346       | 4.7         | 126                    |
| IL15           | P40933         | Interleukin-15                                           | 100                 | 100          | 4.2                | 2.6   | 2.5  | 6.2   | 0.001       | 0.001        | 1263         | 5.9         | 101                    |
| IL17A          | Q16552         | Interleukin-17A                                          | 96                  | 100          | 5.8                | 2.5   | 2.9  | 7.8   | 0.015       | 0.023        | 9231         | 5.6         | 106                    |
| "IL17A  IL17F" | Q16552  Q96PD4 | Interleukin-17A Interleukin-17F                          | 81                  | 77           | 7.6                | 4.5   | 1.2  | 9.6   | 0.008       | 0.074        | 9924         | 5.1         | 104                    |
| IL17B          | Q9UHF5         | Interleukin-17B                                          | 100                 | 100          | 5.8                | 0.9   | 1.5  | 6.8   | 0.005       | 0.042        | 43869        | 6.0         | 108                    |
| IL17C          | Q9P0M4         | Interleukin-17C                                          | 100                 | 100          | 6.9                | 2.6   | 5.0  | 10.4  | 0.017       | 0.067        | 18711        | 5.4         | 112                    |
| IL17F          | Q96PD4         | Interleukin-17F                                          | 79                  | 74           | 7.1                | 9.5   | 0.0  | 14.1  | 16.068      | 51.257       | 72105        | 3.1         | 107                    |
| IL17RA         | Q96F46         | Interleukin-17 receptor A                                | 100                 | 100          | 5.5                | 3.1   | 0.8  | 7.3   | 0.022       | 0.044        | 17298        | 5.6         | 93                     |
| IL17RB         | Q9NRM6         | Interleukin-17 receptor B                                | 98                  | 100          | 4.2                | 1.8   | 0.2  | 5.4   | 0.104       | 0.249        | 93571        | 5.6         | 117                    |
| IL18BP         | O95998         | Interleukin-18-binding protein                           | 100                 | 100          | 4.8                | 3.5   | 0.4  | 6.7   | 5.130       | 26.856       | 2273634      | 4.9         | 102                    |
| IL18R1         | Q13478         | Interleukin-18 receptor 1                                | 100                 | 100          | 5.1                | 2.0   | 1.9  | 6.5   | 0.139       | 0.345        | 58820        | 5.2         | 95                     |
| IL19           | Q9UHD0         | Interleukin-19                                           | 100                 | 100          | 5.0                | 2.8   | 0.8  | 7.4   | 0.029       | 0.047        | 45992        | 6.0         | 107                    |
| IL1B           | P01584         | Interleukin-1 beta                                       | 46                  | 29           | 9.9                | 3.9   | 7.4  | 15.1  | 0.006       | 0.020        | 2021         | 5.0         | 96                     |
| IL1R1          | P14778         | Interleukin-1 receptor type 1                            | 100                 | 100          | 4.8                | 2.3   | 1.4  | 6.3   | 4.270       | 30.956       | 1091798      | 4.5         | 104                    |

Table 2: Performance Validation Data by Target (cont.)

| Target     | UniProt ID    | Protein Name                                                 | Quantifiability (%) |              | AQ Precision (CV%) |       |      |       | LOD (pg/mL) | LLOQ (pg/mL) | ULOQ (pg/mL) | log10 Range | Dilution Linearity (%) |
|------------|---------------|--------------------------------------------------------------|---------------------|--------------|--------------------|-------|------|-------|-------------|--------------|--------------|-------------|------------------------|
| AQ Targets |               |                                                              | Plasma              |              | Plasma             |       |      |       |             |              |              |             |                        |
|            |               |                                                              | Healthy n=57        | Disease n=62 | Intra              | Inter | Lot  | Total |             |              |              |             |                        |
| IL1R2      | P27930        | Interleukin-1 receptor type 2                                | 100                 | 100          | 6.8                | 2.0   | 3.2  | 9.0   | 118.862     | 1017.720     | 3090454      | 3.5         | 100                    |
| IL1RL1     | Q01638        | Interleukin-1 receptor-like 1                                | 100                 | 100          | 8.7                | 5.1   | 5.7  | 13.4  | n/a         | 367.880      | 1108395      | 3.5         | 103                    |
| IL20       | Q9NYY1        | Interleukin-20                                               | 100                 | 100          | 4.9                | 2.1   | 2.0  | 6.7   | 0.012       | 0.026        | 16704        | 5.8         | 98                     |
| IL22       | Q9GZX6        | Interleukin-22                                               | 26                  | 53           | 6.3                | 2.0   | 1.3  | 7.3   | 0.002       | 0.065        | 1228         | 4.3         | 104                    |
| IL27       | Q8NEV9 Q14213 | Interleukin 27 Interleukin-27 subunit beta                   | 96                  | 98           | 5.2                | 3.6   | 0.9  | 7.4   | 0.005       | 0.008        | 4650         | 5.8         | 108                    |
| IL2RB      | P14784        | Interleukin-2 receptor subunit beta                          | 98                  | 100          | 5.5                | 2.6   | 6.5  | 10.2  | 1.088       | 7.939        | 62246        | 3.9         | 97                     |
| IL33       | O95760        | Interleukin 33                                               | 95                  | 100          | 7.2                | 2.8   | 0.2  | 8.3   | 0.007       | 0.040        | 1774         | 4.6         | 116                    |
| IL34       | Q6ZMJ4        | Interleukin-34                                               | 100                 | 100          | 4.7                | 2.7   | 1.1  | 6.4   | 0.025       | 0.231        | 5476         | 4.4         | 77                     |
| IL3RA      | P26951        | Interleukin-3 receptor subunit alpha                         | 98                  | 100          | 5.7                | 2.2   | 2.5  | 7.7   | 2.450       | 24.124       | 175042       | 3.9         | 119                    |
| IL4        | P05112        | Interleukin-4                                                | 12                  | 21           | 7.8                | 1.8   | 2.2  | 9.3   | 0.001       | 0.013        | 783          | 4.8         | 104                    |
| IL4R       | P24394        | Interleukin-4 receptor subunit alpha                         | 98                  | 100          | 7.3                | 4.4   | 0.9  | 9.4   | 7.615       | 143.710      | 275032       | 3.3         | 105                    |
| IL5        | P05113        | Interleukin-5                                                | 100                 | 100          | 4.2                | 2.5   | 0.7  | 5.7   | 0.001       | 0.002        | 2623         | 6.0         | 101                    |
| IL5RA      | Q01344        | Interleukin-5 receptor subunit alpha                         | 98                  | 100          | 7.3                | 2.1   | 0.4  | 8.3   | 10.279      | 179.554      | 1136881      | 3.8         | 103                    |
| IL6        | P05231        | Interleukin-6                                                | 98                  | 100          | 6.2                | 6.2   | 2.0  | 9.6   | 0.029       | 0.057        | 13808        | 5.4         | 106                    |
| IL6R       | P08887        | Interleukin-6 receptor subunit alpha                         | 100                 | 100          | 8.3                | 4.7   | 1.8  | 10.9  | n/a         | 1520.558     | 4525996      | 3.5         | 91                     |
| IL7        | P13232        | Interleukin-7                                                | 100                 | 100          | 5.0                | 0.9   | 3.6  | 6.7   | 0.008       | 0.008        | 1774         | 5.4         | 94                     |
| IL7R       | P16871        | Interleukin-7 receptor subunit alpha                         | 100                 | 100          | 5.9                | 2.9   | 0.1  | 7.0   | 4.679       | 273.369      | 1132406      | 3.6         | 100                    |
| IL9        | P15248        | Interleukin-9                                                | 98                  | 100          | 6.4                | 3.1   | 2.6  | 11.4  | 0.014       | 0.114        | 24520        | 5.3         | 95                     |
| IRAK4      | Q9NWZ3        | Interleukin-1 receptor-associated kinase 4                   | 100                 | 100          | 5.0                | 5.5   | 5.7  | 10.7  | 0.236       | 0.745        | 94454        | 5.1         | 100                    |
| KDR        | P35968        | Vascular endothelial growth factor receptor 2                | 98                  | 98           | 7.0                | 1.0   | 1.1  | 7.8   | 18.855      | 284.088      | 1959998      | 3.8         | 101                    |
| KITLG      | P21583        | Kit ligand                                                   | 98                  | 100          | 6.3                | 7.0   | 20.3 | 22.7  | 1.012       | 1.141        | 2218         | 3.3         | 105                    |
| KLRK1      | P26718        | NKG2-D type II integral membrane protein                     | 100                 | 100          | 5.6                | 2.0   | 1.5  | 6.8   | n/a         | 50.218       | 2421790      | 4.7         | 96                     |
| LAG3       | P18627        | Lymphocyte activation gene 3 protein                         | 100                 | 100          | 5.3                | 2.4   | 1.5  | 6.7   | n/a         | 141.777      | 482571       | 3.5         | 107                    |
| LAMP3      | Q9UQV4        | Lysosomal associated membrane protein 3                      | 98                  | 100          | 6.5                | 2.4   | 4.7  | 9.3   | 2.863       | 2.863        | 34968        | 4.1         | 91                     |
| LGALS9     | O00182        | Galectin-9                                                   | 100                 | 100          | 4.6                | 2.1   | 0.7  | 5.8   | 2.048       | 9.147        | 498923       | 4.7         | 122                    |
| LIF        | P15018        | Leukemia inhibitory factor                                   | 96                  | 100          | 5.5                | 2.3   | 1.2  | 6.9   | 0.012       | 0.050        | 2027         | 4.6         | 101                    |
| LILRB2     | Q8N423        | Leukocyte immunoglobulin-like receptor sub-family B member 2 | 98                  | 100          | 6.5                | 2.4   | 0.0  | 7.5   | 75.248      | 1322.973     | 2005875      | 3.2         | 111                    |
| LTA        | P01374        | Lymphotoxin-alpha                                            | 67                  | 87           | 8.8                | 0.8   | 17.2 | 20.0  | 0.154       | 0.634        | 9367         | 4.2         | 113                    |
| MERTK      | Q12866        | MER proto-oncogene, tyrosine kinase                          | 100                 | 100          | 6.3                | 4.7   | 0.1  | 8.1   | 294.487     | 783.001      | 2903160      | 3.6         | 100                    |
| MICB       | Q29980        | MHC class I polypeptide-related sequence B                   | 98                  | 100          | 4.5                | 5.8   | 1.1  | 8.3   | 7.620       | 7.620        | 2668070      | 5.5         | 108                    |
| MMP12      | P39900        | Macrophage metalloelastase                                   | 98                  | 100          | 7.0                | 2.5   | 0.6  | 8.3   | 4.864       | 11.170       | 450274       | 4.6         | 90                     |
| MMP3       | P08254        | Stromelysin-1                                                | 100                 | 100          | 8.1                | 3.3   | 2.1  | 10.4  | 104.062     | 310.232      | 2299569      | 3.9         | 102                    |
| MMP8       | P22894        | Neutrophil collagenase                                       | 100                 | 100          | 5.2                | 4.3   | 4.6  | 9.1   | 6.050       | 17.259       | 2612126      | 5.2         | 108                    |
| MMP9       | P14780        | Matrix metalloproteinase-9                                   | 100                 | 100          | 5.8                | 3.0   | 11.4 | 15.0  | 73.915      | 459.864      | 1651461      | 3.6         | 76                     |
| NCR1       | O76036        | Natural cytotoxicity triggering receptor 1                   | 98                  | 100          | 5.7                | 1.6   | 7.2  | 11.2  | 3.913       | 6.010        | 19079        | 3.5         | 96                     |
| NGF        | P01138        | Beta-nerve growth factor                                     | 98                  | 100          | 5.6                | 3.2   | 2.3  | 8.7   | 0.486       | 0.734        | 32123        | 4.6         | 90                     |
| OSM        | P13725        | Oncostatin-M                                                 | 100                 | 100          | 5.3                | 4.6   | 2.8  | 8.4   | 0.010       | 0.014        | 4563         | 5.5         | 99                     |
| PDCD1      | Q15116        | Programmed cell death protein 1                              | 98                  | 100          | 6.4                | 5.6   | 5.5  | 11.9  | 1.969       | 13.288       | 33047        | 3.4         | 92                     |
| PDC-D1LG2  | Q9BQ51        | Programmed cell death 1 ligand 2                             | 100                 | 100          | 6.0                | 2.2   | 2.0  | 7.6   | 10.545      | 72.571       | 496763       | 3.8         | 104                    |
| PDGFB      | P01127        | Platelet-derived growth factor subunit B                     | 100                 | 100          | 6.3                | 3.5   | 2.2  | 8.4   | 1.039       | 6.966        | 41889        | 3.8         | 124                    |
| PTX3       | P26022        | Pentraxin-related protein PTX3                               | 98                  | 100          | 6.4                | 4.2   | 7.7  | 12.7  | 3.280       | 42.975       | 244832       | 3.8         | 109                    |
| S100A12    | P80511        | Protein S100-A12                                             | 95                  | 92           | 7.0                | 9.1   | 8.7  | 17.6  | 40.361      | 342.315      | 244388       | 2.9         | 104                    |
| SELE       | P16581        | E-selectin                                                   | 100                 | 100          | 6.4                | 4.5   | 0.5  | 8.4   | 434.219     | 1266.739     | 2559637      | 3.3         | 113                    |

Table 2: Performance Validation Data by Target (cont.)

| Target     | UniProt ID | Protein Name                                               | Quantifiability (%) |              | AQ Precision (CV%) |       |      |       | LOD (pg/mL) | LLOQ (pg/mL) | ULOQ (pg/mL) | log10 Range | Dilution Linearity (%) |
|------------|------------|------------------------------------------------------------|---------------------|--------------|--------------------|-------|------|-------|-------------|--------------|--------------|-------------|------------------------|
| AQ Targets |            |                                                            | Plasma              |              | Plasma             |       |      |       |             |              |              |             |                        |
|            |            |                                                            | Healthy n=57        | Disease n=62 | Intra              | Inter | Lot  | Total |             |              |              |             |                        |
| SIRPA      | P78324     | Tyrosine-protein phosphatase non-receptor type substrate 1 | 98                  | 100          | 6.1                | 2.6   | 1.6  | 7.6   | 481.318     | 1382.975     | 2419646      | 3.2         | 93                     |
| SLAMF1     | Q13291     | Signaling lymphocytic activation molecule                  | 98                  | 100          | 6.2                | 3.2   | 1.9  | 8.1   | 5.295       | 33.603       | 172803       | 3.7         | 93                     |
| TFAA5      | Q7Z5A7     | Chemokine-like protein TFAA-5                              | 86                  | 97           | 4.8                | 1.8   | 1.8  | 6.8   | 14.137      | 60.331       | 91786        | 3.2         | 101                    |
| TEK        | Q02763     | Angiopoietin-1 receptor                                    | 100                 | 100          | 6.6                | 5.0   | 5.6  | 11.1  | 28.718      | 899.919      | 1580840      | 3.2         | 116                    |
| THPO       | P40225     | Thrombopoietin                                             | 100                 | 100          | 7.2                | 4.0   | 1.6  | 9.5   | 3.842       | 16.128       | 704738       | 4.6         | 95                     |
| TIMP2      | P16035     | Metalloproteinase inhibitor 2                              | 4                   | 5            | 8.7                | 1.0   | 23.0 | 25.1  | 51.606      | 642.585      | 360431       | 2.7         | 91                     |
| TLR3       | O15455     | Toll-like receptor 3                                       | 98                  | 100          | 6.2                | 1.9   | 0.6  | 6.9   | 11.444      | 385.002      | 1056123      | 3.4         | 99                     |
| TNFRSF11A  | Q9Y6Q6     | TNF receptor superfamily member 11a                        | 98                  | 100          | 4.7                | 6.7   | 0.7  | 9.1   | 0.348       | 0.563        | 72752        | 5.1         | 104                    |
| TNFRSF13B  | O14836     | Tumor necrosis factor receptor superfamily member 13B      | 100                 | 100          | 6.2                | 2.5   | 0.0  | 7.6   | n/a         | 0.487        | 57798        | 5.1         | 108                    |
| TNFRSF13C  | Q96RJ3     | Tumor necrosis factor receptor superfamily member 13C      | 100                 | 100          | 4.6                | 3.5   | 4.1  | 8.3   | 2.604       | 4.349        | 751571       | 5.2         | 103                    |
| TNFRSF14   | Q92956     | Tumor necrosis factor receptor superfamily member 14       | 100                 | 100          | 6.9                | 3.9   | 0.0  | 8.7   | 15.788      | 239.538      | 486637       | 3.3         | 112                    |
| TNFRSF17   | Q02223     | Tumor necrosis factor receptor superfamily member 17       | 100                 | 100          | 7.3                | 2.8   | 3.3  | 9.6   | 34.922      | 273.497      | 1127218      | 3.6         | 91                     |
| TNFRSF18   | Q9Y5U5     | Tumor necrosis factor receptor superfamily member 18       | 98                  | 100          | 4.9                | 2.6   | 2.3  | 6.8   | 0.073       | 0.301        | 19956        | 4.8         | 95                     |
| TNFRSF1A   | P19438     | Tumor necrosis factor receptor superfamily member 1A       | 98                  | 100          | 7.3                | 1.6   | 2.4  | 8.9   | 81.495      | 165.136      | 667261       | 3.6         | 93                     |
| TNFRSF1B   | P20333     | Tumor necrosis factor receptor superfamily member 1B       | 98                  | 100          | 7.6                | 4.6   | 4.8  | 11.7  | 25.680      | 212.558      | 990786       | 3.7         | 98                     |
| TNFRSF8    | P28908     | Tumor necrosis factor receptor superfamily member 8        | 100                 | 100          | 6.2                | 1.3   | 0.8  | 8.5   | 3.886       | 24.920       | 205129       | 3.9         | 83                     |
| TNFSF10    | P50591     | Tumor necrosis factor ligand superfamily member 10         | 100                 | 100          | 5.7                | 3.6   | 0.9  | 8.4   | 0.602       | 6.273        | 91550        | 4.2         | 116                    |
| TNFSF15    | O95150     | Tumor necrosis factor ligand superfamily member 15         | 100                 | 100          | 6.1                | 5.1   | 1.9  | 8.7   | 0.002       | 0.140        | 21430        | 5.2         | 100                    |
| TNFSF18    | Q9UNG2     | Tumor necrosis factor ligand superfamily member 18         | 100                 | 100          | 3.9                | 1.4   | 0.4  | 4.8   | 0.001       | 0.002        | 1758         | 6.0         | 109                    |
| TNFSF8     | P32971     | Tumor necrosis factor ligand superfamily member 8          | 100                 | 100          | 5.1                | 4.9   | 1.1  | 7.8   | n/a         | 65.464       | 952765       | 4.2         | 108                    |
| TREM1      | Q9NP99     | Triggering receptor expressed on myeloid cells 1           | 100                 | 100          | 6.9                | 6.1   | 5.9  | 12.0  | n/a         | 2.795        | 347970       | 5.1         | 116                    |
| TREM2      | Q9NZC2     | Triggering receptor expressed on myeloid cells 2           | 98                  | 100          | 3.9                | 4.3   | 0.1  | 6.0   | 3.517       | 55.593       | 324812       | 3.8         | 104                    |
| VEGFA      | P15692     | Vascular endothelial growth factor A                       | 100                 | 100          | 5.2                | 3.4   | 9.3  | 14.1  | 53.318      | 53.318       | 142583       | 3.4         | 99                     |
| VEGFC      | P49767     | Vascular endothelial growth factor C                       | 100                 | 100          | 4.8                | 2.4   | 0.8  | 6.2   | 0.259       | 0.460        | 50311        | 5.0         | 104                    |
| VEGFD      | O43915     | Vascular endothelial growth factor D                       | 21                  | 35           | 7.6                | 2.0   | 21.2 | 23.0  | 5.147       | 55.911       | 12450        | 2.3         | 99                     |
| VSNL1      | P62760     | Visinin-like protein 1                                     | 100                 | 100          | 5.2                | 3.0   | 0.1  | 7.0   | 0.123       | 0.203        | 35599        | 5.2         | 111                    |
| VSTM1      | Q6UX27     | V-set and transmembrane domain containing 1                | 81                  | 87           | 5.3                | 3.4   | 0.9  | 6.9   | 0.205       | 8.575        | 677          | 1.9         | 99                     |
| WNT7A      | O00755     | Protein Wnt-7a                                             | 79                  | 90           | 5.5                | 2.9   | 2.2  | 7.2   | 0.041       | 0.626        | 34584        | 4.7         | 120                    |

## Ordering Information

### NULISaseq Assay Panels

| Product Name                    | Plate Format | Sample Type  | Catalog Number |
|---------------------------------|--------------|--------------|----------------|
| NULISaseq Inflammation Panel AQ | 96           | plasma/serum | 800154         |

### 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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