NULISAseq™ Neuro 220 Panel

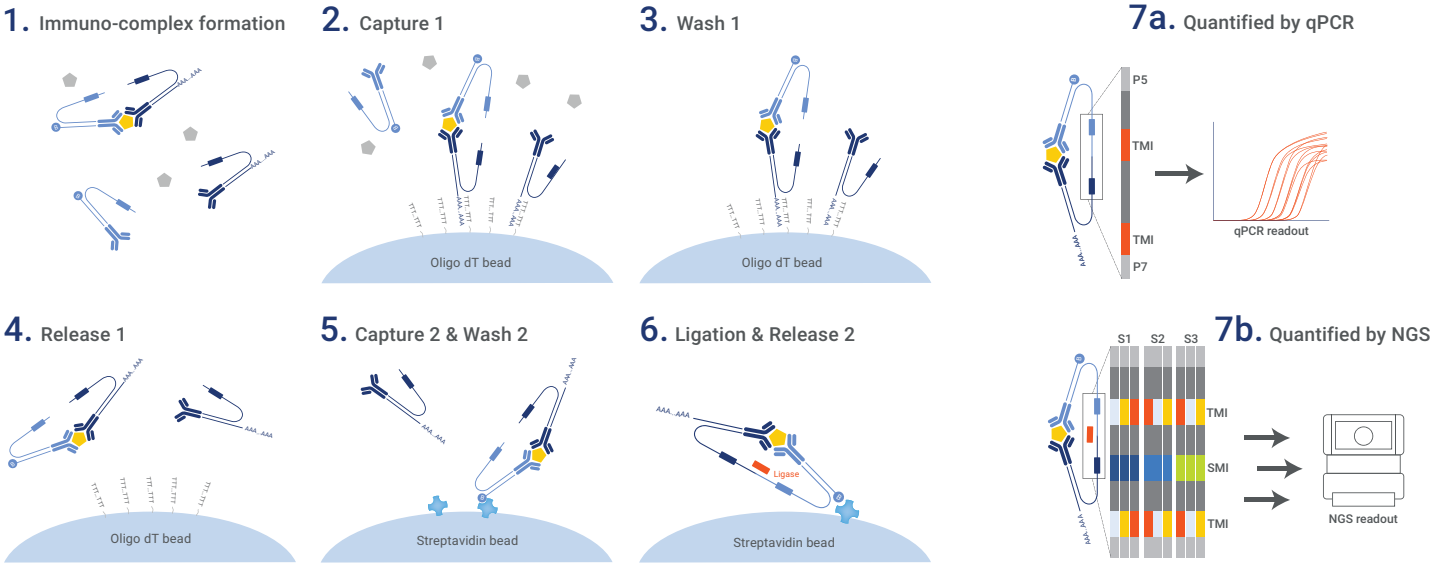
DATA SHEET

Introduction

Neurodegenerative Disease research is complicated by a high degree of clinical heterogeneity and the prevalence of co-pathologies. The identification of clinically relevant biomarkers for neurological disorders is critical in the development of better tools for diagnosis, disease progression and prediction of therapeutic outcomes. The NULISAseq Neuro 220 Panel offers comprehensive coverage of the Hallmarks of Neurodegenerative Disease with highly sensitive and specific multiplexed analysis of 220 biomarkers using only 25 µL of sample, providing researchers with an automated solution to dissect disease heterogeneity, uncover co-pathologies and identify signatures of disease prognosis, progression and therapeutic response in clinical cohorts.

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 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.



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NULISAseq™ Neuro 220 Panel

Sensitivity & Detectability

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. These values are rescaled and log-transformed to obtain LOD in NPQ. A maximum of one outlier NC value may be omitted. Median and interquartile ranges (IQR) for LOD were calculated across 26 runs (Table 3).

Target detectability is the percentage of samples above LOD. Detectability was assessed in 37 normal and 135 disease EDTA plasma samples. More than 94% of targets, excluding reverse curve targets and targets with rare isoforms, were detectable in at least 50% of samples (Table 3, Figure 1).

Neuro 220 Detectability: Normal and Disease Plasma Samples

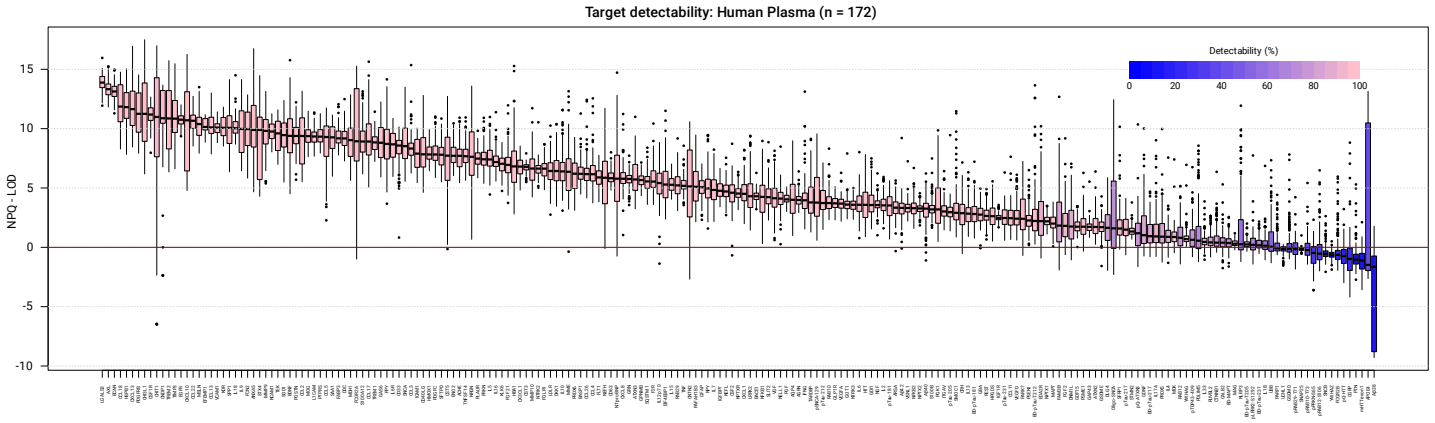

Figure 1. Detectability was assessed in 37 normal and 135 disease EDTA plasma samples. More than 94% of targets were detectable in at least 50% of samples. Data courtesy of Carlos Cruchaga, PhD, WashU Medicine, Neurogenomics and Informatics Center

Data Normalization & Calculation of NULISA Protein Quantification (NPQ) Values

NULISA Protein Quantification (NPQ) units are log2-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 inter-plate controls (IPCs) on the plate. Data is then rescaled and log2-transformed to obtain the data in NPQ, which are approximately normally distributed values amenable to downstream statistical analysis.

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Precision

Intra-assay precision, or repeatability, was measured to assess the variation in technical replicates within the same assay run; this is reported in Tables 1-3 as intra-plate coefficient of variation (CV). Inter-assay precision was measured to assess the variation in technical replicates across different assay runs or on different days; this is reported in Tables 1-3 as inter-plate CV. For each target, CV was determined using a variance component analysis model to assess the contribution of various factors to the total CV of the normalized reads. Six plasma and six CSF samples with 3 technical replicates each were measured across a set of 12 runs which included 2 reagent lots and 2 instruments across 6 days. Variance component analysis models were fit for each sample and target and included the factors reagent lot, instrument, inter-plate, and intra-plate (Tables 1-3). For each target, the estimated CV for each component was averaged across the 6 samples. Values below LOD and samples with average raw read counts below 100 were excluded from CV calculations.

Table 1: Across-target Median Coefficients of Variation

CV component	PLASMA	CSF
Intra-plate	6.73	8.90
Inter-plate	4.28	5.03

Table 2: Percentage of Targets with CV Below 30%

CV component	PLASMA	CSF
Intra-plate	99%	99%
Inter-plate	97%	98%

Cross-Reactivity

To assess cross-reactivity, targets were randomly assigned to two sets of 26 pools containing either four or five targets each, such that no two targets shared a pool for both sets.

Counts were normalized using an internal control. Cross-reactivity for each target was quantified as (maximum non-target pool count –background)/(average target pool counts – background) * 100, where the background was calculated as the median count across non-target pools. Cross reactivity to proteins outside the panel that have high homology to targeted proteins was also assessed.

Cross-reactivity was only assessed for targets with commercially available recombinant antigen. Cross-reactivity data for each target is reported in Table 3, Figure 2.

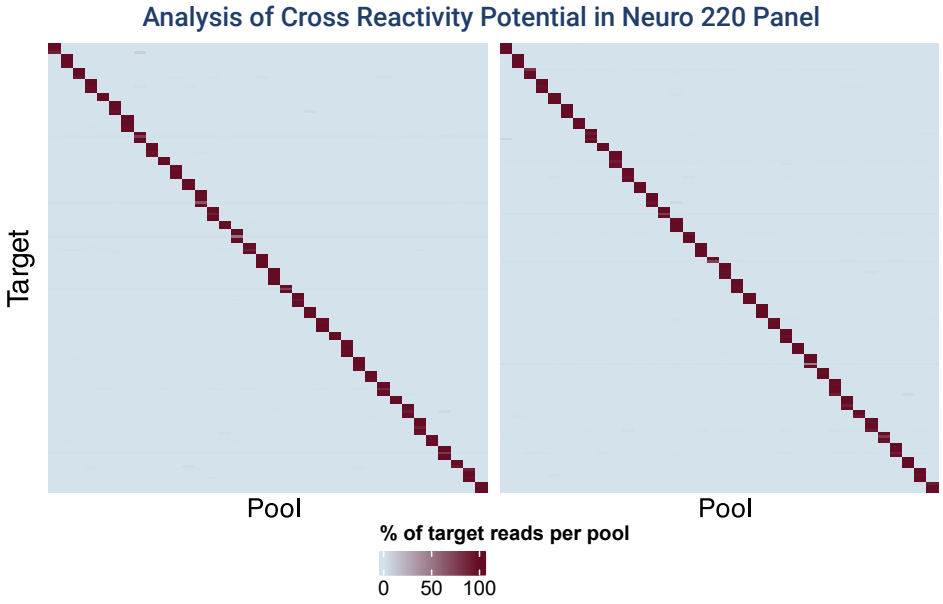

Figure 2. Heatmaps for each pool set show the percentage of target reads occurring in assigned target pools, on the diagonal, or non-target pools, in the off-diagonal cells. Most of the 162 targets have cross-reactivity < 1%.

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Table 3: Performance Validation Data by Target. Targets added to Neuro 220 Panel, respective to the CNS 120 Panel, are highlighted in bold text.

Target	UniProt ID	Protein Name	Detectability (%)		CV (%)				LOD (NPQ) Median (IQR)	Cross Reactivity
			Plasma normal n=37	Plasma disease n=135	Plasma		CSF			
					Intra	Inter	Intra	Inter		
Abeta38	P05067	Amyloid-beta precursor protein	41.86	100	n/a	n/a	8.2	10.6	7.3 (1.5, 8.4)	< 1%
Abeta40	P05067	Amyloid-beta precursor protein	67.44	97.87	16.8	10.9	6.8	11.1	5.7 (0.0, 7.0)	< 1%
Abeta42	P05067	Amyloid-beta precursor protein	58.41	97.87	12.4	19.4	7.9	11.7	11.0 (10.7, 11.3)	< 1%
ACHE	P22303	Acetylcholinesterase	100.0	100.0	6.9	2.2	11.2	2.1	4.2 (3.1, 4.6)	n/a
AGRN	O00468	Agrin	100.0	100.0	7.3	5.3	4.8	6.0	9.4 (9.2, 9.7)	n/a
ANXA5	P08758	Annexin A5	100.0	100.0	4.6	4.1	9.1	10.5	1.8 (1.2, 2.3)	n/a
APOA1	**P02647**	**Apolipoprotein A-I**	**n/a**	**n/a**	**4.5**	**1.2**	**6.5**	**3.5**	**n/a** [i]	**n/a**
APOA2	**P02652**	**Apolipoprotein A-II**	**n/a**	**n/a**	**5.1**	**5.2**	**4.9**	**3.8**	**n/a** [i]	**n/a**
APOE	P02649	Apolipoprotein E	n/a	n/a	6.3	0.8	3.4	0.8	n/a [i]	< 1%
APOE4	P02649	Apolipoprotein E	24.3	43.0	5.8	1.0	7.1	5.8	0.0 (0.0, 0.6)	< 1%
APOH	**P02749**	**Beta-2-glycoprotein 1**	**n/a**	**n/a**	**12.0**	**15.7**	**4.8**	**7.0**	**n/a** [i]	**n/a**
AQP4	**P55087**	**Aquaporin-4**	**100.0**	**100.0**	**8.1**	**14.0**	**4.7**	**8.0**	**7.8 (7.6, 8.1)**	**n/a**
ARSA	P15289	Arylsulfatase A	97.30	100.0	4.2	5.0	8.1	3.5	9.5 (9.3, 9.6)	< 1%
ATXN2	**Q99700**	**Ataxin 2**	**100.0**	**100.0**	**5.8**	**6.3**	**10.8**	**3.2**	**10.2 (9.9, 10.7)**	**n/a**
ATXN3	**P54252**	**Ataxin 3**	**100.0**	**100.0**	**4.6**	**9.3**	**8.8**	**8.9**	**7.0 (6.8, 7.8)**	**< 1%**
AXL	**P30530**	**AXL receptor tyrosine kinase**	**100.0**	**100.0**	**5.3**	**3.4**	**9.3**	**1.6**	**0.0 (0.0, 0.0)**	**n/a**
BACE1	P56817	Beta-secretase 1	100.0	100.0	6.2	3.5	4.7	5.2	9.3 (9.0, 9.5)	n/a
BASP1	P80723	Brain abundant membrane attached signal protein 1	100.0	100.0	32.5	45.9	69.4	85.6	7.3 (6.7, 8.3)	n/a
BCAN	**Q96GW7**	**Brevican core protein**	**100.0**	**100.0**	**11.8**	**8.9**	**5.7**	**10.3**	**0.0 (0.0, 0.0)**	**n/a**
BD-MAPT	P10636	Microtubule-associated protein tau (Brain-Derived)	81.1	77.8	7.9	4.2	10.7	3.8	12.5 (12.4, 12.6)	< 1%
BD-pTau-181	P10636	Microtubule-associated protein tau (Brain-Derived)	100.0	100.0	8.0	9.4	9.8	4.7	11.2 (10.6, 11.6)	< 1%
BD-pTau-205	**P10636**	**Microtubule-associated protein tau (Brain-Derived)**	**86.5**	**83.7**	**7.1**	**5.5**	**5.4**	**4.3**	**12.6 (12.4, 12.7)**	**n/a**
BD-pTau-212	**P10636**	**Microtubule-associated protein tau (Brain-Derived)**	**100.0**	**100.0**	**6.5**	**4.0**	**5.5**	**2.2**	**11.7 (10.8, 11.8)**	**n/a**
BD-pTau-217	P10636	Microtubule-associated protein tau (Brain-Derived)	83.8	88.9	12.2	8.6	9.6	1.1	8.4 (8.1, 8.6)	< 1%
BD-pTau-231	P10636	Microtubule-associated protein tau (Brain-Derived)	62.2	68.1	5.8	6.4	4.3	5.7	13.2 (13.1, 13.2)	n/a
BDNF	P23560	Brain-derived neurotrophic factor	100.0	100.0	6.2	6.8	n/a	n/a	3.2 (0.0, 4.9)	< 1%
C1q	**P02745, P02746, P02747**	**Complement C1q and subunits A, B, C**	**n/a**	**n/a**	**11.4**	**9.5**	**6.6**	**10.1**	**n/a** [i]	**< 1%**
CALB2	P22676	Calretinin	75.7	78.5	20.2	17.0	8.8	2.1	7.2 (6.8, 7.5)	n/a
CCL11	P51671	Eotaxin	100.0	100.0	13.4	8.1	n/a	n/a	6.6 (6.1, 6.9)	n/a
CCL13	Q99616	C-C motif chemokine 13	100.0	100.0	5.0	3.2	15.0	3.0	0.0 (0.0, 3.0)	< 1%
CCL17	Q92583	C-C motif chemokine 17	100.0	100.0	7.0	1.4	14.8	0.0	0.0 (0.0, 4.5)	n/a
CCL18	P55774	C-C motif chemokine 18	100.0	100.0	4.9	2.3	n/a	n/a	0.0 (0.0, 0.0)	n/a
CCL2	P13500	C-C motif chemokine 2	100.0	100.0	6.6	3.7	5.2	3.5	3.2 (0.0, 4.3)	n/a
CCL22	O00626	C-C motif chemokine 22	100.0	100.0	5.6	2.9	13.8	6.5	0.0 (0.0, 1.7)	n/a
CCL26	Q9Y258	C-C motif chemokine 26	100.0	100.0	6.2	2.0	14.1	3.4	6.0 (5.7, 6.3)	n/a
CCL3	P10147	C-C motif chemokine 3	100.0	100.0	4.4	2.5	4.1	2.4	3.4 (3.0, 3.8)	294.3% [a]
CCL4	P13236	C-C motif chemokine ligand 4	100.0	100.0	9.9	3.9	19.3	0.0	0.0 (0.0, 4.5)	1438.1% [b]
CCL5	**P13501**	**C-C motif chemkine 18**	**100.0**	**100.0**	**9.0**	**5.3**	**n/a**	**n/a**	**3.8 (3.2, 4.2)**	**n/a**
CD33	**P20138**	**Myeloid cell surface antigen CD33**	**100.0**	**100.0**	**7.7**	**3.2**	**13.3**	**4.2**	**5.1 (4.2, 5.6)**	**n/a**
CD40LG	P29965	CD40 ligand	100.0	100.0	6.3	3.9	n/a	n/a	0.0 (0.0, 0.0)	n/a
CD63	P08962	CD63 antigen	100.0	100.0	4.4	1.8	6.9	1.5	6.8 (6.7, 6.9)	< 1%
CGRP	**P06881**	**Calcitonin gene-related peptide 1**	**18.9**	**25.2**	**25.4**	**40.6**	**28.5**	**24.8**	**9.2 (8.8, 9.7)**	**n/a**
CHI3L1	P36222	Chitinase-3-like protein 1	100.0	100.0	6.4	2.3	4.6	2.4	6.1 (5.2, 6.7)	< 1%
CHIT1	Q13231	Chitotriosidase-1	95.35	100.0	8.9	3.9	9.5	3.8	0.0 (0.0, 0.0)	< 1%
CNDP1	**Q96KN2**	**Beta-Ala-His dipeptidase**	**90.7**	**89.36**	**5.9**	**6.5**	**7.3**	**7.2**	**0.0 (0.0, 0.0)**	**< 1%**

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Target	UniProt ID	Protein Name	Detectability (%)		CV (%)				LOD (NPQ) Median (IQR)	Cross Reactivity
					Plasma		CSF			
			Plasma normal n=37	Plasma disease n=135	Intra	Inter	Intra	Inter		
CNTN2	Q02246	Contactin-2	100.0	98.5	n/a	n/a	5.8	6.9	0.0 (0.0, 0.0)	n/a
CRH	P06850	Corticoliberin	97.3	99.3	13.3	7.2	7.8	10.7	8.9 (8.7, 9.4)	n/a
CRP	P02741	C-reactive protein	n/a	n/a	8.7	3.0	3.2	15.8	n/a [i]	< 1%
CSF1R	**P07333**	**Macrophase colony-stimulating factor 1 receptor**	**100.0**	**100.0**	**6.4**	**3.1**	**8.5**	**4.0**	**0.0 (0.0, 0.0)**	**n/a**
CSF2	P04141	Granulocyte-macrophage colony-stimulating factor	97.3	100.0	6.5	4.7	7.7	2.7	9.8 (9.2, 10.1)	n/a
CST3	P01034	Cystatin-C	100.0	100.0	5.2	3.6	4.3	5.3	6.5 (6.2, 6.9)	n/a
CST5	**P28325**	**Cystatin-D**	**100**	**99.3**	**6.9**	**32.7**	**n/a**	**n/a**	**5.9 (4.6, 6.8)**	**n/a**
CTNNB1	**P35222**	**Catenin beta-1**	**73.0**	**84.4**	**6.9**	**8.0**	**8.7**	**3.5**	**12.4 (12.2, 12.6)**	**n/a**
CX3CL1	P78423	Fractalkine	100.0	100.0	5.2	4.3	6.7	4.7	6.0 (5.7, 6.5)	n/a
CXCL1	P09341	Growth-regulated alpha protein	100.0	95.74	6.5	14.9	10.7	15.4	8.4 (8.1, 8.6)	< 1%
CXCL10	P02778	C-X-C motif chemokine 10	100.0	100.0	8.0	2.9	5.8	5.9	0.0 (0.0, 0.0)	n/a
CXCL13	**O43927**	**C-X-C motif chemokine 13**	**100.0**	**100.0**	**6.0**	**2.9**	**14.2**	**4.2**	**0.0 (0.0, 0.0)**	**n/a**
CXCL8	P10145	Interleukin-8, IL8	100.0	100.0	5.0	11.3	3.8	10.7	7.5 (7.2, 7.9)	< 1%
DDC	P20711	Aromatic-L-amino-acid decarboxylase	100.0	100.0	3.6	2.3	8.0	1.6	2.9 (2.6, 3.1)	n/a
DKK1	**O94907**	**Dickkopf-related protein 1**	**100.0**	**100.0**	**6.6**	**8.0**	**16.1**	**10.0**	**7.2 (6.6, 7.7)**	**< 1%**
DLG4	**P78352**	**Disks large homolog4**	**73.0**	**86.7**	**9.5**	**9.2**	**10.9**	**6.3**	**11.3 (10.1, 11.5)**	**n/a**
DNM1L	**O00429**	**Dynamin 1 like**	**81.1**	**91.9**	**9.1**	**7.7**	**12.3**	**7.5**	**7.7 (7.3, 8.0)**	**n/a**
EDA2R	**Q9HAV5**	**Tumor necrosis factor receptor superfamily member 27**	**86.5**	**91.9**	**14.3**	**5.3**	**14.3**	**8.8**	**8.3 (7.9, 8.6)**	**n/a**
EFEMP1	**Q12805**	**EGF-containing fibulin-like extracellular matrix protein 1**	**100.0**	**100.0**	**6.8**	**0.2**	**10.3**	**0.4**	**0.0 (0.0, 0.0)**	**n/a**
EGFR	**P00533**	**Epidermal growth factor receptor**	**100.0**	**100.0**	**6.7**	**4.3**	**16.6**	**6.6**	**0.0 (0.0, 0.0)**	**n/a**
EIF4EBP1	**Q13541**	**Eukaryotic translation initiation factor 4E-binding protein 1**	**100.0**	**100.0**	**6.6**	**5.7**	**16.4**	**10.3**	**8.9 (8.3, 9.3)**	**n/a**
ENO2	P09104	Gamma-enolase	100.0	100.0	4.5	5.2	5.0	4.3	4.0 (3.1, 5.3)	n/a
F2R	**P25116**	**Coagulation factor II thrombin receptor**	**100.0**	**100.0**	**7.5**	**5.9**	**15.3**	**3.9**	**7.6 (1.5, 8.5)**	**n/a**
FABP3	P05413	Fatty acid-binding protein, heart	100.0	100.0	5.9	2.4	5.3	3.3	3.2 (2.4, 3.8)	n/a
FAM3B	**P58499**	**Protein FAM3B**	**83.8**	**85.2**	**10.9**	**5.0**	**10.9**	**6.2**	**7.3 (6.7, 7.7)**	**< 1%**
FCGR2A	**P12318**	**Low affinity immunoglobulin gamma Fc region receptor II-a**	**100.0**	**98.5**	**10.5**	**4.2**	**27.9**	**7.5**	**0.0 (0.0, 0.0)**	**n/a**
FCGR2B	**P31994**	**Low affinity immunoglobulin gamma Fc region receptor II-b**	**21.6**	**19.3**	**17.1**	**15.5**	**8.8**	**21.0**	**9.4 (8.9, 9.7)**	**n/a**
FCN2	Q15485	Ficolin-2	100.0	100.0	8.1	9.1	n/a	n/a	0.0 (0.0, 0.0)	< 1%
FGF2	P09038	Fibroblast growth factor 2	94.6	95.6	8.0	4.3	8.9	5.7	8.8 (8.7, 8.9)	n/a
FGF21	**Q9NSA1**	**Fibroblast growth factor 21**	**100.0**	**100.0**	**4.6**	**3.2**	**15.4**	**0.0**	**4.5 (4.1, 5.1)**	**n/a**
FGFBP1	**Q14512**	**Fibroblast growth factor-binding protein 1**	**n/a**	**n/a**	**11.1**	**4.6**	**n/a**	**n/a**	**10.9 (10.5, 11.3)**	**n/a**
FLT1	P17948	Vascular endothelial growth factor receptor 1	100.0	100.0	6.4	3.4	8.7	6.0	8.3 (8.0, 8.4)	n/a
FOLR1	P15328	Folate receptor alpha	100.0	100.0	8.1	2.4	6.6	2.9	2.1 (0.0, 3.1)	n/a
GAP43	**P17677**	**Neuromodulin**	**100.0**	**100.0**	**7.9**	**4.7**	**11.0**	**12.1**	**10.8 (10.4, 11.5)**	**n/a**
GAS6	**Q14393**	**Growth arrest-specific protein 6**	**100.0**	**100.0**	**9.8**	**6.4**	**12.7**	**7.6**	**0.0 (0.0, 0.0)**	**n/a**
GBA1	**P04062**	**Glucosylceramidase beta**	**100.0**	**99.3**	**7.0**	**8.0**	**11.2**	**7.3**	**7.7 (7.4, 8.0)**	**< 1%**
GDF15	Q99988	Growth/differentiation factor 15	100.0	100.0	5.2	4.6	5.5	1.9	8.9 (8.5, 12.4)	n/a
GDI1	P31150	Rab GDP dissociation inhibitor alpha	100.0	97.87	7.0	5.2	8.6	4.9	8.9 (8.4, 9.3)	n/a
GDNF	P39905	Glial cell line-derived neurotrophic factor	78.4	88.1	6.0	1.4	8.5	1.6	9.4 (9.2, 9.5)	< 1%
GFAP	P14136	Glial fibrillary acidic protein	100.0	100.0	7.8	2.5	4.6	3.1	6.7 (5.9, 7.3)	n/a
GLP1R	**P43220**	**Glucagon-like peptide 1 receptor**	**100.0**	**100.0**	**8.8**	**5.0**	**7.7**	**4.5**	**8.5 (8.1, 8.8)**	**n/a**
GOT1	P17174	Aspartate aminotransferase, cytoplasmic	100.0	100.0	6.4	1.0	7.3	2.7	8.0 (7.7, 8.3)	< 1%
GPNMB	**Q14956**	**Transmembrane glycoprotein NMB**	**100.0**	**100.0**	**7.8**	**6.1**	**18.2**	**12.2**	**6.5 (5.9, 7.3)**	**n/a**
GRN	**P28799**	**Progranulin**	**100.0**	**100.0**	**6.3**	**3.0**	**19.1**	**10.5**	**7.3 (6.8, 7.5)**	**n/a**

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			Plasma normal n=37	Plasma disease n=135	Plasma		CSF			
					Intra	Inter	Intra	Inter		
GSDME	**O60443**	**Gasdermin E**	**100.0**	**98.5**	**13.1**	**15.2**	**13.5**	**7.4**	**9.8 (9.3, 10.4)**	**n/a**
HBA1	P69905	Hemoglobin subunit alpha	100.0	100.0	5.3	2.9	12.3	4.9	1.6 (1.1, 2.0)	< 1%
HMOX1	**P09601**	**Heme oxygenase 1**	**100.0**	**100.0**	**7.6**	**4.2**	**n/a**	**n/a**	**2.7 (0.0, 5.7)**	**n/a**
HPGDS	**O60760**	**Hematopoietic prostaglandin D synthase**	**100.0**	**100.0**	**8.9**	**7.3**	**14.3**	**1.9**	**9.9 (9.4, 10.3)**	**n/a**
HSPB1	**P04792**	**Heat Shock protein beta-1**	**100.0**	**100.0**	**9.9**	**3.4**	**20.3**	**0.0**	**0.0 (0.0, 0.7)**	**< 1%**
HTT	P42858	Huntingtin	100.0	100.0	5.4	3.3	8.1	5.1	8.8 (8.5, 9.1)	< 1%
ICAM1	P05362	Intercellular adhesion molecule 1	100.0	100.0	8.4	3.9	n/a	n/a	0.0 (0.0, 0.0)	n/a
IFNG	P01579	Interferon gamma	100.0	100.0	6.2	3.3	11.6	30.6	8.9 (8.4, 9.4)	n/a
IGF1R	P08069	Insulin-like growth factor 1 receptor	100.0	100.0	5.1	1.4	4.9	1.0	11.4 (11.4, 11.5)	n/a
IGFBP7	Q16270	Insulin-like growth factor-binding protein 7	100.0	100.0	7.2	3.7	5.9	5.1	8.1 (7.8, 8.7)	n/a
IL10	P22301	Interleukin-10	100.0	100.0	6.9	2.2	11.5	1.5	6.2 (6.0, 6.6)	n/a
IL12p70	P29459_ P29460	Interleukin-12 subunit beta_Interleukin-12 subunit alpha	100.0	99.3	7.3	2.7	15.3	6.2	8.4 (8.0, 8.8)	n/a
IL13	P35225	Interleukin-13	100.0	100.0	6.4	10.4	7.0	8.7	10.0 (9.9, 10.2)	n/a
IL15	P40933	Interleukin-15	100.0	100.0	6.3	4.1	5.4	4.2	8.3(7.9, 8.7)	n/a
IL16	Q14005	Pro-interleukin-16	100.0	100.0	4.5	2.2	8.7	2.0	4.1 (3.7, 5.6)	n/a
IL17A	Q16552	Interleukin-17A	97.3	90.4	7.0	3.8	7.4	5.5	10.7 (10.5, 10.8)	n/a
IL18	Q14116	Interleukin-18	100.0	100.0	3.9	3.3	15.4	4.0	2.9 (1.4, 3.5)	n/a
IL1B	P01584	Interleukin-1 beta	51.4	64.4	10.0	3.0	16.7	0.0	5.3 (4.9, 5.8)	n/a
IL2	P60568	Interleukin-2	100.0	100.0	8.8	9.0	11.2	9.7	6.7 (6.4, 7.1)	n/a
IL33	O95760	Interleukin 33	91.9	89.6	10.0	8.0	15.0	8.2	7.2 (6.9, 7.8)	n/a
IL4	P05112	Interleukin-4	86.5	89.6	13.6	13.9	18.3	7.9	7.0 (6.7, 7.3)	n/a
IL5	P05113	Interleukin-5	100.0	100.0	9.7	13.2	6.7	13.3	6.3 (5.5, 6.7)	n/a
IL6	P05231	Interleukin-6	100.0	100.0	6.5	2.9	5.4	1.9	8.9 (8.5, 9.3)	n/a
IL6R	P08887	Interleukin-6 receptor subunit alpha	100.0	100.0	7.2	7.6	n/a	n/a	0.0 (0.0, 4.8)	n/a
IL7	P13232	Interleukin-7	100.0	100.0	6.0	6.6	11.2	4.6	8.7 (8.2, 8.8)	n/a
IL9	P15248	Interleukin-9	100.0	100.0	7.5	3.8	6.7	1.7	7.9 (0.0, 8.2)	n/a
ITGAV	**P06756**	**Integrin subunit alpha V**	**100.0**	**100.0**	**6.3**	**6.2**	**10.3**	**18.0**	**9.8 (9.5, 10.2)**	**n/a**
KDR	P35968	Vascular endothelial growth factor receptor 2	100.0	100.0	9.1	0.4	15.6	2.2	0.0 (0.0, 0.0)	< 1%
KLK6	Q92876	Kallikrein-6	100.0	100.0	11.2	6.1	4.8	8.9	7.1 (6.3, 7.9)	< 1%
L1CAM	**P32004**	**Neural cell adhesion molecule L1**	**100.0**	**100.0**	**5.5**	**2.5**	**14.1**	**4.1**	**3.8 (2.4, 4.8)**	**n/a**
LDLR	**P01130**	**Low-density lipoprotein receptor**	**100.0**	**100.0**	**8.5**	**4.0**	**20.5**	**2.1**	**5.8 (0.0, 6.5)**	**n/a**
LGALS3	**P17931**	**Galectin-3**	**100.0**	**100.0**	**6.9**	**3.5**	**n/a**	**n/a**	**0.0 (0.0, 0.0)**	**< 1%**
LRRK2	**Q5S007**	**Leucine-rich repeat serine/threonine-protein kinase 2**	**100.0**	**100.0**	**6.0**	**10.4**	**14.2**	**9.5**	**9.1 (8.6, 9.9)**	**< 1%**
MAG	**P20916**	**Myelin associated glycoprotein**	**94.6**	**90.4**	**4.2**	**4.1**	**3.8**	**5.0**	**13.1 (13.0, 13.2)**	**n/a**
MDH1	P40925	Malate dehydrogenase, cytoplasmic	100.0	100.0	4.8	2.6	8.9	6.7	3.1 (1.9, 3.7)	n/a
MDK	**P21741**	**Midkine**	**97.3**	**95.6**	**6.5**	**5.0**	**7.4**	**7.4**	**12.8 (12.5, 12.9)**	**n/a**
mHTT-exon1	**P42858**	**Huntingtin**	**2.7**	**12.6**	**n/a**	**n/a**	**n/a**	**n/a**	**10.5 (10.1, 11.1)**	**< 1%**
MME	P08473	Membrane metalloendopeptidase	97.3	100.0	8.5	5.3	n/a	n/a	2.2 (0.0, 4.0)	n/a
MMP10	**P09238**	**Stromelysin-2**	**100.0**	**100.0**	**6.7**	**5.9**	**14.9**	**7.1**	**11.7 (8.5, 11.8)**	**n/a**
MMP9	**P14780**	**Matrix metalloproteinase-9**	**100.0**	**100.0**	**5.5**	**1.4**	**7.9**	**5.1**	**4.0 (3.3, 4.5)**	**< 1%**
MOG	**Q16653**	**Myelin-oligodendrocyte glycoprotein**	**100.0**	**100.0**	**7.5**	**4.7**	**4.0**	**6.8**	**0.0 (0.0, 5.3)**	**n/a**
MSLN	Q13421	Mesothelin	100.0	100.0	4.6	3.8	11.9	3.0	5.8 (4.5, 6.5)	n/a
NCAM1	**P13591**	**Neural cell adhesion molecule 1**	**100.0**	**100.0**	**5.6**	**0.6**	**6.4**	**0.9**	**0.0 (0.0, 0.0)**	**n/a**
NEFH	P12036	Neurofilament heavy polypeptide	100.0	99.3	6.3	3.1	5.1	3.2	10.6 (10.5, 10.7)	< 1%
NEFL (NfL)	P07196	Neurofilament light polypeptide	100.0	100.0	6.1	3.9	3.7	4.5	10.6 (10.5, 10.8)	n/a
NELL1	**Q92832**	**Protein kinase C-binding protein NELL1**	**100.0**	**100.0**	**10.3**	**8.6**	**16.9**	**8.6**	**8.9 (8.7, 9.2)**	**< 1%**

NULISAseq™ Neuro 220 Panel

Table 3: Performance Validation Data by Target. Targets added to Neuro 220 Panel, respective to the CNS 120 Panel, are highlighted in bold text.

Target	UniProt ID	Protein Name	Detectability (%)		CV (%)				LOD (NPQ) Median (IQR)	Cross Reactivity
					Plasma		CSF			
			Plasma normal n=37	Plasma disease n=135	Intra	Inter	Intra	Inter		
NFKB1	**P19838**	**Nuclear factor NF-kappa-B p105 subunit**	**100.0**	**100.0**	**6.0**	**6.3**	**18.7**	**0.0**	**8.3 (7.5, 8.7)**	**n/a**
NFKB2	**Q00653**	**Nuclear factor kappa B subunit2**	**100.0**	**100.0**	**8.0**	**9.0**	**14.5**	**13.4**	**8.7 (8.1, 10.0)**	**n/a**
NGF	P01138	Beta-nerve growth factor	100.0	100.0	5.3	1.8	8.7	1.4	10.2 (10.1, 10.4)	n/a
NLRP3	**Q96P20**	**NLR family pyrin domain containing 3**	**51.4**	**61.5**	**5.9**	**4.5**	**5.7**	**5.2**	**10.0 (9.9, 10.2)**	**n/a**
NPTX1	Q15818	Neuronal pentraxin-1	100.0	100.0	6.4	1.7	3.6	3.8	11.3 (11.2, 11.5)	< 1%
NPTX2	P47972	Neuronal pentraxin-2	100.0	100.0	5.8	2.3	4.7	2.3	10.6 (10.3, 10.8)	n/a
NPTXR	Q95502	Neuronal pentraxin receptor	100.0	100.0	7.9	5.2	6.1	6.3	8.8 (8.5, 9.0)	n/a
NPY	P01303	Neuropeptide Y	100.0	100.0	11.3	8.6	11.4	7.4	6.0 (5.8, 6.2)	n/a
NRGN	Q92686	Neurogranin	100.0	100.0	6.2	6.9	17.3	10.0	7.7 (7.4, 7.8)	n/a
NT-proBNP	**P16860**	**N-terminal prohormone of bain natriuretic peptide**	**100.0**	**99.3**	**10.5**	**4.8**	**18.7**	**10.0**	**6.2 (5.9, 6.6)**	**< 1%**
NTRK2	**Q16620**	**BDNF/NT-3 growth factors receptor**	**100.0**	**100.0**	**6.7**	**5.2**	**10.2**	**12.2**	**7.9 (7.7, 8.2)**	**n/a**
NTRK3	**Q16288**	**NT-3 growth factor receptor**	**100.0**	**100.0**	**7.1**	**3.4**	**8.4**	**6.4**	**9.5 (9.2, 10.5)**	**n/a**
Oligo-SNCA	P37840	Alpha-synuclein	67.6	76.3	12.8	10.7	27.3	19.0	2.9 (2.0, 3.4)	99.3% [c]
PAFAH1B3	**Q15102**	**Platelet-activating factor acetylhydrolase IB subunit gamma**	**100.0**	**100.0**	**4.2**	**4.0**	**16.1**	**2.6**	**7.8 (7.6, 8.3)**	**n/a**
PARK7	Q99497	Protein/nucleic acid deglycase DJ-1	94.6	98.5	8.5	21.5	14.4	7.7	9.3 (8.9, 9.8)	< 1%
PARP1	**P09874**	**Poly [ADP-ribose} polymerase 1**	**37.8**	**45.9**	**5.2**	**5.9**	**5.0**	**4.1**	**9.1 (8.9, 11.0)**	**n/a**
PDGFC	**Q9NRA1**	**Platelet-derived growth factor C**	**100.0**	**100.0**	**5.2**	**4.6**	**12.0**	**3.4**	**6.5 (6.0, 6.9)**	**n/a**
PDGFRB	P09619	Platelet-derived growth factor receptor beta	100.0	100.0	6.3	3.0	14.7	3.2	0.0 (0.0, 1.8)	n/a
PDLIM5	Q96HC4	PDZ and LIM domain 5	81.1	68.1	8.2	5.3	6.2	0.5	8.4 (8.3, 8.6)	n/a
PGF	P49763	Placenta growth factor	100.0	100.0	5.2	2.4	5.3	2.0	9.7 (9.6, 9.9)	n/a
PGK1	P00558	Phosphoglycerate kinase 1	100.0	100.0	4.6	4.3	11.4	12.7	10.2 (9.7, 10.4)	22.7% [d]
PLAUR	**Q03405**	**Urokinase plaminogen activator surface receptor**	**100.0**	**100.0**	**5.1**	**2.8**	**7.3**	**3.0**	**5.8 (5.3, 6.2)**	**n/a**
pLR-RK2-S1292	**Q5S007**	**Leucine-rich repeat serine/threonine-protein kinase 2**	**56.8**	**68.1**	**22.9**	**29.2**	**26.7**	**21.7**	**10.8 (10.5, 11.7)**	**n/a**
POSTN	Q15063	Periostin	100.0	100.0	8.4	4.0	n/a	n/a	0.0 (0.0, 0.0)	n/a
PPBP	**P02775**	**Platelet basic protein**	**n/a**	**n/a**	**6.4**	**1.0**	**5.6**	**5.2**	**n/a [i]**	**n/a**
pPRKN-S65	**O60260**	**E3 ubiquitin-protein ligase parkin (Parkin)**	**27.0**	**28.9**	**21.3**	**130.2**	**12.3**	**22.0**	**9.3 (8.7, 9.6)**	**10.1% [e]**
PPY	**P01298**	**Pancreatic prohormone**	**100.0**	**100.0**	**7.9**	**4.0**	**n/a**	**n/a**	**6.7 (5.5, 7.2)**	**n/a**
pQ-ATXN3	**P54252**	**Ataxin-3**	**78.4**	**79.3**	**9.3**	**23.0**	**30.4**	**31.9**	**8.6 (8.2, 9.0)**	**5.1% [f]**
pQ-HTT	**P42858**	**Huntingtin**	**5.4**	**20.7**	**17.9**	**54.3**	**19.9**	**57.0**	**10.5 (10.0, 11.2)**	**1.8% [g]**
pRAB10-T73	**P61026**	**Ras-related protein Rab-10**	**24.3**	**41.5**	**21.4**	**27.5**	**23.6**	**22.8**	**10.0 (9.7, 10.5)**	**< 1%**
pR-AB12-S106	**Q61Q22**	**Ras-related protein Rab-12**	**21.6**	**37.0**	**52.2**	**17.4**	**18.4**	**21.2**	**11.6 (9.3, 11.8)**	**< 1%**
pRAB29-T71	**O14966**	**Ras-related protein Rab-7L1**	**37.8**	**39.3**	**18.9**	**116.6**	**23.9**	**26.8**	**6.1 (5.6, 6.40**	**< 1%**
PRDX5	**P30044**	**Peroxiredoxin-5, mitochondrial**	**81.1**	**85.9**	**8.9**	**9.8**	**21.3**	**18.7**	**10.0 (9.7, 11.5)**	**n/a**
PRDX6	P30041	Peroxiredoxin-6	100.0	100.0	4.8	6.8	10.1	4.2	5.9 (5.6, 6.0)	n/a
PRKN	**O60260**	**E3 ubiquitin-protein ligase parkin**	**100.0**	**100.0**	**4.9**	**3.8**	**5.6**	**4.7**	**10.3 (5.0, 10.4)**	**< 1%**
PROS1	**P07225**	**Vitamin K-dependent protein S**	**n/a**	**n/a**	**6.0**	**1.5**	**4.7**	**1.8**	**n/a [i]**	**n/a**
PSEN1	P49768	Presenilin 1	100.0	100.0	9.5	10.8	15.9	11.5	8.4 (7.7, 8.7)	n/a
PSME1	**Q06323**	**Proteassome activator complex subunit1**	**97.3**	**100.0**	**7.2**	**3.0**	**14.2**	**5.0**	**9.4 (8.0, 10.0)**	**n/a**
pSNCA-S129	P37840	Alpha-synuclein	100.0	100.0	6.1	3.5	11.1	10.8	9.5 (9.4, 9.7)	< 1%
	Q13148	TAR DNA-binding protein 43	100.0	100.0	3.4	2.8	4.1	2.6	8.6 (8.4, 8.8 0	< 1%
pTau-181	P10636	Microtubule-associated protein tau	100.0	100.0	6.2	4.9	7.4	2.4	10.2 (9.8, 10.5)	< 1%
pTau-205	**P10636**	**Microtubule-associated protein tau**	**100.0**	**100.0**	**6.5**	**7.9**	**5.8**	**5.7**	**9.4 (9.0, 10.5)**	**< 1%**
pTau-212	**P10636**	**Microtubule-associated protein tau**	**100.0**	**100.0**	**8.6**	**5.7**	**5.6**	**3.6**	**8.5 (8.0, 9.1)**	**< 1%**
pTau-217	P10636	Microtubule-associated protein tau	100.0	97.0	13.6	8.1	8.2	0.2	7.7 (6.9, 8.2)	< 1%
pTau-231	**P10636**	**Microtubule-associated protein tau**	**100.0**	**100.0**	**5.2**	**4.9**	**3.3**	**5.9**	**9.7 (9.5, 10.6)**	**< 1%**

NULISAseq™ Neuro 220 Panel

Table 3: Performance Validation Data by Target. Targets added to Neuro 220 Panel, respective to the CNS 120 Panel, are highlighted in bold text.

Target	UniProt ID	Protein Name	Detectability (%)		CV (%)				LOD (NPQ) Median (IQR)	Cross Reactivity
			Plasma normal n=37	Plasma disease n=135	Plasma		CSF			
					Intra	Inter	Intra	Inter		
pTDP43-409	**Q13148**	**TAR DNA-binding protein 43**	**73.0**	**80.7**	**7.7**	**13.5**	**10.9**	**8.7**	**13.0 (12.7, 13.2)**	**< 1%**
PTN	P21246	Pleiotrophin	5.4	9.6	12.1	8.1	20.5	11.5	5.1 (4.8, 5.4)	n/a
PTPRS	**Q13332**	**Receptor-type tyrosine-protein phosphate S**	**100.0**	**100.0**	**4.6**	**2.9**	**9.3**	**2.7**	**4.1 (3.7, 4.6)**	**n/a**
RAB10	**P161026**	**RAB10, member RAS oncogene family**	**100**	**100.0**	**6.1**	**4.2**	**8.4**	**6.9**	**9.4 (9.2, 9.6)**	**< 1%**
RAB12	**Q6IQ22**	**RAB12, member RAS oncogene family**	**62..2**	**83.7**	**14.4**	**17.9**	**28.9**	**16.9**	**10.0 (9.4, 10.5)**	**< 1%**
RAB29	**O14966**	**RAB29, member RAS oncogene family**	**100.0**	**100.0**	**5.4**	**2.4**	**10.3**	**2.4**	**6.7 (6.3, 7.1)**	**< 1%**
REST	Q13127	RE1 silencing transcription factor	100.0	100.0	6.0	3.6	13.4	6.1	10.1 (9.8, 10.2)	n/a
RUVBL2	Q9Y230	RuvB like AAA ATPase2	78.4	86.7	3.8	3.2	4.2	1.4	10.2 (10.1, 11.1)	n/a
S100A12	P80511	Protein S100-A12	100.0	100.0	6.6	9.2	10.4	12.4	5.1 (4.5, 5.5)	< 1%
S100B	P04271	S100 calcium binding protein B	100.0	100.0	6.3	4.8	5.7	2.6	7.5 (7.2, 7.6)	n/a
SAA1	P0DJI8	Serum amyloid A-1 protein	100.0	100.0	3.4	1.0	18.2	9.0	0.0 (0.0, 0.0)	< 1%
SERPINA3	**P01011**	**Alpha-1-antichymotrypsin**	**n/a**	**n/a**	**6.8**	**3.4**	**2.7**	**3.8**	**n/a** [i]	**< 1%**
SFRP1	Q8N474	Secreted frizzled-related protein 1	100.0	96.3	7.1	10.4	7.4	8.3	12.6 (12.5, 12.8)	n/a
SFTPD	P35247	Pulmonary surfactant-associated protein D	100.0	100.0	7.0	3.5	11.7	2.5	6.4 (6.2, 6.7)	n/a
SLIT2	O94813	Slit homolog 2 protein	100.0	100.0	6.7	3.8	12.0	6.1	9.9 (9.6, 10.0)	< 1%
SMOC1	Q9H4F8	SPARC-related modular calcium-binding protein 1	100.0	100.0	9.5	4.2	20.9	11.1	7.9 (7.5, 8.2)	n/a
SNAP25	P60880	Synaptosomal-associated protein 25	10.8	5.2	3.5	0.7	3.8	1.5	13.2 (13.1, 13.2)	n/a
SNCA	P37840	Alpha-synuclein	100.0	100.0	4.7	3.8	12.6	6.2	4.4 (3.6, 5.0)	< 1%
SNCB	Q16143	Synuclein beta	5.4	16.3	16.2	33.7	11.1	3.6	10.0 (9.8, 10.1)	n/a
SOD1	P00441	Superoxide dismutase [Cu-Zn]	100.0	100.0	6.4	3.0	4.2	3.2	5.9 (5.3, 6.2)	< 1%
SPP1	**P10451**	**Osteopontin**	**100.0**	**100.0**	**8.2**	**6.3**	**5.8**	**6.7**	**5.5 (4.8, 6.0)**	**< 1%**
SQSTM1	Q13501	Sequestosome-1	100.0	100.0	7.6	3.2	9.3	3.1	5.5 (5.1, 6.1)	n/a
STMN2	**Q93045**	**Stathmin-2**	**100.0**	**100.0**	**6.4**	**5.4**	**3.9**	**4.5**	**9.2 (9.0, 9.4)**	**< 1%**
STX4	**Q12846**	**Syntaxin-4**	**100.0**	**100.0**	**5.5**	**3.5**	**16.8**	**0.2**	**4.7 (3.4, 5.1)**	**n/a**
TARDBP (TDP43)	Q13148	TAR DNA-binding protein 43	100.0	100.0	3.4	2.8	4.1	2.6	8.6 (8.4, 8.8)	n/a
TEK	Q02763	Angiopoietin-1 receptor	100.0	100.0	5.0	2.3	15.3	5.7	2.0 (0.0, 3.2)	n/a
TIMP3	P35625	Metalloproteinase inhibitor 3	100.0	100.0	5.7	6.1	16.3	5.4	0.0 (0.0, 5.0)	< 1%
TNF	P01375	Tumor necrosis factor	100.0	100.0	6.0	3.6	15.7	3.6	8.8 (8.2, 9.1)	n/a
TNFSF14	**O43557**	**Tumor necrosis factor ligand superfamily member 14**	**100.0**	**100.0**	**4.3**	**3.3**	**22.0**	**0.0**	**4.1 (3.5, 4.9)**	**n/a**
TREM1	Q9NP99	Triggering receptor expressed on myeloid cells 1	100.0	100.0	5.5	3.7	14.7	5.2	6.5 (5.9, 7.3)	n/a
TREM2	Q9NZC2	Triggering receptor expressed on myeloid cells 2	100.0	100.0	6.0	1.9	6.8	3.0	0.0 (0.0, 0.0)	n/a
tTau (MAPT)	P10636	Microtubule-associated protein tau	100.0	100.0	7.9	4.7	8.4	1.5	8.9 (8.4, 9.7)	< 1%
UBB	P0CG47	Polyubiquitin-B	45.9	54.8	5.5	3.3	4.1	2.9	11.4 (11.3, 11.4)	n/a
UCHL1	P09936	Ubiquitin carboxyl-terminal hydrolase isozyme L1	23.3	31.9	1.1	0.0	6.2	2.9	12.4 (12.3, 12.6)	n/a
VCAM1	P19320	Vascular cell adhesion protein 1	100.0	100.0	5.4	4.1	16.7	5.0	0.0 (0.0, 0.0)	n/a
VEGFA	P15692	Vascular endothelial growth factor A	100.0	100.0	4.8	3.1	4.3	2.5	8.1 (7.9, 8.3)	< 1%
VEGFD	O43915	Vascular endothelial growth factor D	100.0	100.0	8.7	2.8	9.8	3.8	10.4 (10.3, 10.6)	n/a
VGF	O15240	VGF nerve growth factor inducible	100.0	100.0	6.8	5.8	5.2	4.4	8.3 (7.9, 9.0)	n/a
VSNL1	P62760	Visinin-like protein 1	100.0	100.0	8.0	5.6	4.8	5.1	9.4 (9.0, 9.6)	9.9% [h]
YWHAG	P61981	Tyrosine 3-monooxygenase/tryptophan 5-monooxy-genase activation protein gamma	100.0	100.0	7.2	3.8	5.8	2.3	11.7 (11.4, 11.9)	< 1%
YWHAZ	P63104	14-3-3 protein zeta/delta	2.7	2.2	8.2	6.1	10.9	0.0	8.9 (8.7, 9.0)	n/a

a. Cross-reactivity with CCL3L1, which has 95% homology with CCL3. CCL3L1 is not in the Neuro 220 panel.
b. Cross-reactivity with CCL4L1, which has 97% homology with CCL4. CCL4L1 is not in the Neuro 220 panel.
c. Cross-reactivity with pSNCA, < 1% with unphosphorylated SNCA Ag. Potential aggregation of the pSNCA antigen during testing has not been assessed.
d. Cross-reactivity with PGK2, which has 88% homology with PGK1. PGK2 is not in the Neuro 220 panel.
e. Cross-reactivity with PRKN, but affinity against p-PRKN-S65 much higher.
f. Cross-reactivity with ATXN3. Due to uniqueness of this target (polyQ-length mutation), 1% criteria may not apply in this situation.
g. Cross-reactivity with HTT. Due to uniqueness of this target (polyQ-length mutation), 1% criteria may not apply in this situation.
h. Cross-reactivity with HPCAL4, which has 89% homology with VSNL1. HPCAL4 is not in the Neuro 220 panel.
i. Reported only for plasma/serum samples due to target concentration.

NULISAseq™ Neuro 220 Panel

Ordering Information

NULISAseq Panel			
Product Name	**Plate Format**	**Sample Type**	**Catalog Number**
NULISAseq Neuro 220	96	plasma/serum/CSF	800471

Related Panels			
Product Name	**Plate Format**	**Sample Type**	**Catalog Number**
NULISAseq Inflammation Panel	96	plasma/serum	800103
NULISAseq Inflammation Panel AQ	96	plasma/serum	800154

Consumables & Buffers		
Product Name	**Qty**	**Catalog Number**
NULISA 10X Wash Buffer	1L	801056

Instrument		
Product Name	**Qty**	**Catalog Number**
Alamar ARGO HT System	1	800101

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