NULISAseq™ Mouse Panel 120

DATA SHEET

Introduction

Mouse and other murine models are indispensable tools in translational clinical research to study disease mechanisms and therapeutic interventions. Inflammation, neurodegeneration, and oncogenesis are central mechanisms in the development and treatment of many diseases. The NULISAseq Mouse Panel 120 measures the presence and relative quantitation of 120+ cytokines, chemokines, immune-related, and neurology-focused proteins simultaneously from less than 25 µL sample input. This panel offers the most comprehensive coverage of protein biomarkers in murine models, leveraging advanced multiplexing technology to deliver high sensitivity and throughput. 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 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. More details on the assay protocol can be found here.

Data Normalization and Quantification Methods

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.

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.

Validation data LOD median and interquartile ranges (IQR) summarize LODs generated across 24 runs (Table 3). Target detectability is the percentage of samples above LOD. Detectability was assessed in 68 EDTA plasma (30 healthy and 38 disease), 68 serum (30 healthy and 38 disease) and 27 CSF samples. More than 99% of targets were detectable in at least 50% of plasma or serum samples, and more than 76% of targets were detectable in at least 50% of CSF samples (Table 3, Figure 1).

A maximum of one outlier NC value may be omitted.

Mouse Panel Detectability

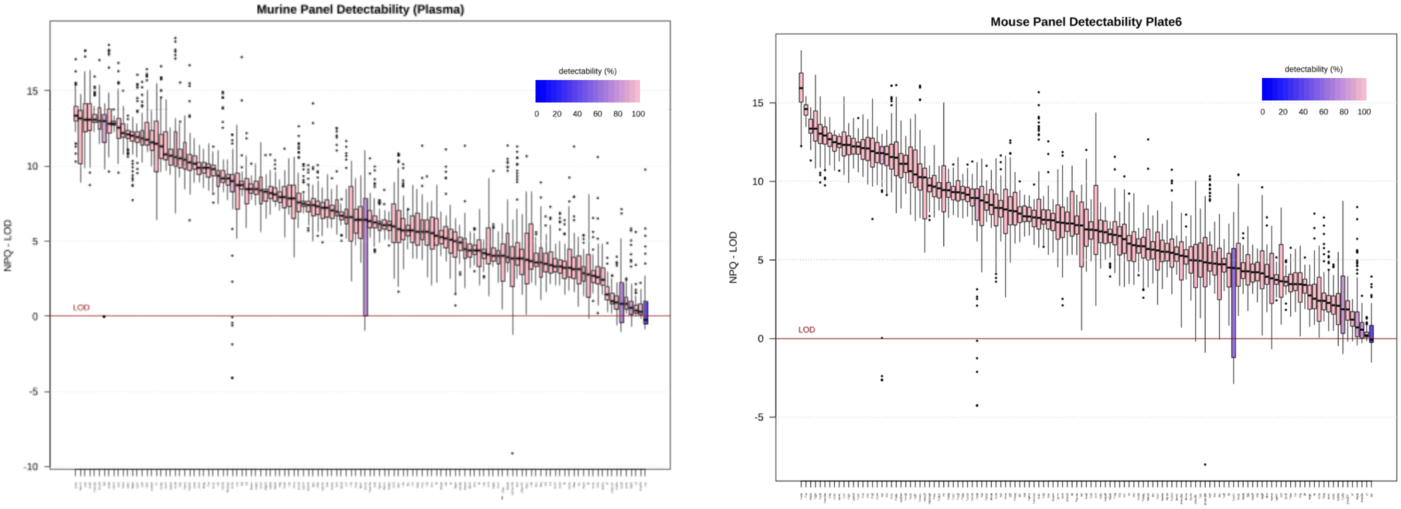

Figure 1. Detectability was assessed in healthy & disease EDTA plasma (left) and serum (right) samples. More than 99% of targets were detectable in at least 50% of samples.

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, or reproducibility, 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 samples with 3 technical replicates each were measured across a set of 15 runs which included 2 reagent lots and 2 instruments across 8 days (Tables 1-3). For each target, the estimated CV for each component was averaged across the 6 samples. Values below LOD were excluded from CV calculations.

CV component	Median CV (%)
Intra-plate	5.16
Inter-plate	4.95
Bay	1.62
Instrument	1.58
Reagent Lot	1.10
Total	9.60

Table 1: Across-target median Coefficients of Variation

CV component	% Targets < 30%
Intra-plate	100%
Inter-plate	100%
Total	97.5%

Table 2: Percentage of targets with CV below 30%

Cross Reactivity

To assess cross-reactivity, each target was tested either as a single antigen or randomly assigned to various subpools. 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. Cross-reactivity was only assessed for targets with commercially available recombinant antigen (Table 3).

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Table 3: Performance Validation Data by Target

Target	UniProt ID	Protein Name	Detectability (%)					CV (%)			LOD (NPQ) Median (IQR)	Cross Reactivity
			Plasma		Serum		CSF	Plasma				
			Healthy n=30	Disease n=38	Healthy n=30	Disease n=38	Combined n=27	Intra	Inter	Total		
Angpt2	O35608	Angiopoietin-2	100	100	100	100	4	4.7	7.7	21.0	4.2 (4.0, 4.5)	<1%
Bace1	P56818	Beta-secretase 1	100	100	100	100	100	7.9	9.1	12.6	8.0 (7.8, 8.3)	<1%
Btc	Q05928	Betacellulin	100	100	100	100	100	6.7	2.6	8.3	7.8 (7.2, 8.2)	<1%
Casp3	P70677	Caspase-3	100	100	100	100	100	7.3	5.4	10.8	3.3 (2.8, 3.6)	<1%
Ccl11	P48298	C-C motif chemokine 11	100	100	100	100	100	5.1	6.0	8.2	4.5 (4.2, 4.8)	<1%
Ccl12	Q62401	C-C motif chemokine 12	80	100	90	100	100	4.0	4.4	11.4	4.5 (4.3, 4.9)	<1%
Ccl17	Q9WUZ6	C-C motif chemokine 17	100	100	100	100	0	7.2	7.8	13.1	9.9 (9.6, 10.2)	<1%
Ccl19	O70460	C-C motif chemokine 19	100	100	100	100	100	5.3	4.9	8.5	0.0 (0.0, 0.0)	<1%
Ccl2	P10148	C-C motif chemokine 2	100	100	100	100	100	5.1	7.5	11.5	3.3 (0.0, 4.4)	<1%
Ccl20	O89093	C-C motif chemokine 20	83	61	93	47	7	6.5	4.1	16.8	8.9 (8.8, 9.0)	NA
Ccl24	Q9JKC0	C-C motif chemokine 24	100	100	100	100	100	6.0	9.1	15.3	0.0 (0.0, 4.9)	<1%
Ccl27	Q9Z1X0	C-C motif chemokine 27	100	100	100	100	11	6.2	9.2	12.2	0.0 (0.0, 0.0)	NA
Ccl3	P10855	C-C motif chemokine 3	100	100	100	100	100	5.2	3.6	6.9	3.7 (3.3, 4.2)	<1%
Ccl4	P14097	C-C motif chemokine 4	100	100	100	100	100	5.2	4.6	8.9	0.0 (0.0, 3.6)	<1%
Ccl5	P30882	C-C motif chemokine 5	100	100	100	100	100	4.0	5.5	8.1	6.4 (6.2, 6.8)	<1%
Ccl7	Q03366	C-C motif chemokine 7	100	100	100	100	100	3.9	3.8	6.6	0.0 (0.0, 0.3)	<1%
Ccn4	O54775	CCN family member 4	100	100	100	100	100	4.4	4.4	6.5	0.0 (0.0, 0.1)	<1%
Cd27	P41272	CD27 antigen	100	100	100	100	100	4.8	4.0	7.0	0.0 (0.0, 1.0)	<1%
Cd274	Q9EP73	Programmed cell death 1 ligand 1	100	100	100	100	89	2.7	4.4	6.6	5.2 (5.0, 5.4)	<1%
Cd40l	P27548	CD40 ligand	100	100	100	100	100	4.4	4.6	7.6	5.1 (4.7, 5.5)	<1%
Cd63	P41731	CD63 antigen	100	100	100	100	100	4.6	4.3	11.3	3.3 (2.9, 3.5)	<1%
Cdh1	P09803	Cadherin-1	100	100	100	100	100	3.5	4.5	7.5	0.2 (0.0, 0.3)	<1%
Chi3l1	Q61362	Chitinase-3-like protein 1	100	100	100	100	100	4.5	4.8	11.6	0.0 (0.0, 0.3)	<1%
Chit1	Q9D7Q1	Chitotriosidase-1	100	100	100	100	30	5.2	3.2	8.3	0.5 (0.0, 2.6)	<1%
Crp	P14847	C-reactive protein	100	100	100	100	NA	8.4	0.8	16.5	22.1 (21.6, 26.6)	<1%
Csf1	P07141	Macrophage colony-stimulating factor 1	100	100	100	100	100	4.9	7.5	22.3	4.5 (4.2, 4.7)	<1%
Csf2	P01587	Granulocyte-macrophage colony-stimulating factor	100	100	100	100	100	4.4	4.3	7.6	7.7 (7.4, 8.2)	<1%
Csf3	P09920	Granulocyte colony-stimulating factor	100	100	100	100	7	4.8	3.2	6.7	5.2 (4.9, 5.6)	<1%
Ctla4	P09793	Cytotoxic T-lymphocyte protein 4	100	100	100	100	15	4.0	5.7	10.4	9.2 (9.0, 9.3)	<1%
Cx3cl1	O35188	Fractalkine	100	100	100	100	100	5.7	4.5	7.9	5.3 (1.8, 5.9)	<1%
Cxcl1	P12850	C-X-C motif chemokine 1	100	100	100	100	100	4.9	4.1	7.4	0.0 (0.0, 0.7)	<1%
Cxcl11	Q9JHH5	C-X-C motif chemokine 11	80	71	80	61	52	6.1	6.0	9.7	5.9 (5.3, 6.7)	NA
Cxcl13	O55038	C-X-C motif chemokine 13	100	100	100	100	37	4.6	5.4	14.7	8.3 (7.6, 8.8)	<1%
Cxcl16	Q8BSU2	C-X-C motif chemokine 16	100	100	100	100	100	5.1	3.1	6.4	3.4 (0.0, 3.6)	<1%
Cxcl2	P10889	C-X-C motif chemokine 2	100	100	100	100	100	4.1	3.7	5.9	4.5 (2.9, 4.9)	<1%
Cxcl5	P50228	C-X-C motif chemokine 5	100	100	100	100	100	5.9	5.6	11.1	0.3 (0.0, 1.4)	<1%
Cxcl9	P18340	C-X-C motif chemokine 9	100	100	100	100	96	7.2	7.8	25.4	0.0 (0.0, 0.0)	<1%
Dll1	Q61483	Delta-like protein 1	100	100	100	100	96	6.1	6.3	10.8	0.0 (0.0, 0.5)	<1%
Eno2	P17183	Gamma-enolase	100	100	100	100	100	4.4	8.6	46.4	5.7 (5.4, 6.7)	<1%
Epcam	Q99JW5	Epithelial cell adhesion molecule	100	100	100	100	100	4.7	4.4	10.6	5.3 (5.1, 5.5)	<1%
Epo	P07321	Erythropoietin	100	100	100	100	100	4.6	4.4	7.1	5.0 (4.8, 5.3)	<1%
Flt3lg	P49772	Fms-related tyrosine kinase 3 ligand	100	100	100	100	100	4.8	3.6	7.9	2.6 (2.2, 3.0)	<1%
Gdf15	Q9Z0J7	Growth/differentiation factor 15	100	100	100	100	96	3.6	5.5	10.0	4.4 (4.2, 4.6)	<1%
Gfap	P03995	Glial fibrillary acidic protein	100	100	100	100	100	6.1	4.9	9.3	8.3 (8.0, 8.5)	<1%

Table 3: Performance Validation Data by Target (cont.)

Target	UniProt ID	Protein Name	Detectability (%)					CV (%)			LOD (NPQ) Median (IQR)	Cross Reactivity
			Plasma		Serum		CSF	Plasma				
			Healthy n=30	Disease n=38	Healthy n=30	Disease n=38	Combined n=27	Intra	Inter	Total		
Gpnmb	Q99P91	Transmembrane glycoprotein NMB	100	100	100	100	100	6.4	5.7	9.1	5.6 (5.4, 5.9)	<1%
Gzmb	P04187	Granzyme B	100	100	100	100	15	12.0	6.9	17.8	9.1 (8.2, 9.4)	<1%
Havcr2	Q8VIM0	Hepatitis A virus cellular receptor 2 homolog	100	100	100	100	100	5.9	6.8	10.6	0.0 (0.0, 0.0)	<1%
Hgf	Q08048	Hepatocyte growth factor	100	76	100	79	100	5.9	3.9	15.6	0.0 (0.0, 0.0)	<1%
Icam1	P13597	Intercellular adhesion molecule 1	100	100	100	100	100	4.0	3.5	6.5	0.0 (0.0, 0.0)	<1%
Ifna1	P01572	Interferon alpha-1	100	100	100	100	59	8.3	3.1	10.3	0.0 (0.0, 0.0)	24.5% (ifna5)
Ifna2	P01573	Interferon alpha-2	100	100	100	100	100	3.9	4.1	9.6	2.8 (2.6, 3.3)	4.2% (ifna11)
Ifng	P01580	Interferon gamma	100	100	100	100	22	8.8	2.5	10.5	5.2 (4.8, 5.5)	<1%
Il10	P18893	Interleukin-10	100	100	100	100	85	3.9	5.3	14.1	6.8 (6.6, 7.1)	<1%
Il11	P47873	Interleukin-11	100	100	100	100	7	5.5	7.6	10.8	5.3 (5.2, 6.0)	<1%
Il12b	P43432	Interleukin-12 subunit beta	100	100	100	100	100	5.0	3.9	7.1	4.8 (4.7, 5.0)	<1%
Il13	P20109	Interleukin-13	100	100	100	100	100	4.4	5.5	14.8	6.0 (5.6, 6.4)	<1%
Il15	P48346	Interleukin-15	100	100	100	100	93	5.3	6.5	9.1	8.9 (8.7, 9.1)	<1%
Il16	O54824	Interleukin-16	100	100	100	100	78	5.4	5.9	8.7	0.8 (0.0, 3.1)	<1%
Il17a	Q62386	Interleukin-17A	100	100	100	100	41	7.0	3.8	8.8	3.5 (0.0, 4.1)	<1%
Il17b	Q9QXT6	Interleukin-17B	100	100	100	84	15	11.6	7.0	16.7	8.9 (8.6, 9.6)	<1%
Il17f	Q7TNI7	Interleukin-17F	100	100	100	100	15	7.4	3.7	9.8	4.0 (3.6, 4.4)	<1%
Il18	P70380	Interleukin-18	100	100	100	100	100	4.7	6.4	15.1	4.0 (3.4, 4.3)	<1%
Il19	Q8CJ70	Interleukin-19	100	100	100	100	93	6.6	5.2	11.7	5.8 (5.3, 6.3)	<1%
Il1a	P01582	Interleukin-1 alpha	100	100	100	100	96	4.5	3.2	19.2	3.7 (3.6, 4.2)	<1%
Il1b	P10749	Interleukin-1 beta	100	100	100	100	48	6.5	6.0	12.2	6.1 (5.5, 6.2)	<1%
Il1rl1	P14719	Interleukin-1 receptor-like 1	100	100	100	100	96	4.2	4.6	9.5	0.1 (0.0, 0.5)	<1%
Il2	P04351	Interleukin-2	100	100	100	100	59	5.6	5.9	10.0	8.9 (8.6, 9.0)	<1%
Il22	Q9JJY9	Interleukin-22	100	100	100	100	100	4.3	5.2	7.8	0.2 (0.0, 2.2)	<1%
Il27a	Q8K3I6	Interleukin-27 subunit alpha	100	100	100	100	89	4.1	7.0	8.3	4.3 (3.8, 4.5)	<1%
Il2ra	P01590	Interleukin-2 receptor subunit alpha	100	100	100	100	4	9.0	8.2	18.7	7.1 (7.0, 7.6)	<1%
Il31	Q6EAL8	Interleukin-31	37	42	40	37	22	6.1	4.7	14.8	9.9 (9.8, 10.1)	<1%
Il33	Q8BVZ5	Interleukin-33	100	100	100	100	81	5.4	7.3	9.7	9.2 (9.0, 9.6)	<1%
Il4	P07750	Interleukin-4	100	100	100	100	100	5.6	2.3	7.8	7.1 (6.7, 7.4)	<1%
Il5	P04401	Interleukin-5	100	100	100	100	48	5.4	2.8	8.7	7.7 (7.6, 7.8)	<1%
Il6	P08505	Interleukin-6	100	97	100	92	70	5.2	4.4	8.0	9.0 (8.9, 9.3)	<1%
Il7	P10168	Interleukin-7	100	100	100	97	0	12.2	5.8	34.1	8.4 (8.2, 8.7)	<1%
Il9	P15247	Interleukin-9	100	100	100	97	74	6.5	3.5	8.2	4.9 (4.4, 5.2)	<1%
Kitlg	P20826	Kit ligand	100	100	100	100	100	6.0	5.4	10.6	0.0 (0.0, 2.2)	<1%
Lgals1	P16045	Galectin-1	100	100	100	100	96	3.9	5.1	14.1	9.0 (8.8, 9.5)	NA
Lgals3	P16110	Galectin-3	100	100	100	100	100	4.1	4.1	6.5	0.0 (0.0, 1.4)	<1%
Lgmn	O89017	Legumain	100	100	100	100	93	5.7	13.7	23.6	4.9 (4.5, 5.2)	<1%
Lif	P09056	Leukemia inhibitory factor	100	100	97	92	100	4.2	1.4	6.5	12.4 (12.4, 12.6)	<1%
Mif	P34884	Macrophage migration inhibitory factor	100	100	100	100	100	5.0	3.9	32.2	6.9 (6.6, 7.5)	<1%
Mmp8	O70138	Neutrophil collagenase	100	100	100	100	22	3.5	4.9	8.5	3.6 (3.3, 4.1)	<1%
Nampt	Q99KQ4	Nicotinamide phosphoribosyltransferase	100	100	100	97	100	12.1	11.0	22.9	8.3 (7.7, 8.6)	<1%
Nefh	P19246	Neurofilament heavy polypeptide	100	100	100	100	100	8.3	7.1	13.5	6.3 (5.6, 6.8)	NA
Nefl	P08551	Neurofilament light polypeptide	100	100	100	100	100	5.0	4.0	7.9	7.3 (7.0, 7.4)	<1%
Nptx2	O70340	Neuronal pentraxin-2	100	100	100	100	100	6.1	3.7	8.0	8.2 (8.1, 8.3)	<1%

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			Plasma		Serum		CSF	Plasma				
			Healthy n=30	Disease n=38	Healthy n=30	Disease n=38	Combined n=27	Intra	Inter	Total		
Pdcd1lg2	Q9WUL5	Programmed cell death 1 ligand 2	100	100	100	100	100	4.6	2.1	6.3	0.0 (0.0, 3.2)	<1%
Pdgfa	P20033	Platelet-derived growth factor subunit A	100	100	100	97	19	4.6	3.2	7.2	7.5 (7.3, 7.7)	<1%
Pdgfb	P31240	Platelet-derived growth factor subunit B	100	100	100	100	52	6.8	8.1	14.4	0.0 (0.0, 0.2)	<1%
Pgf	P49764	Placenta growth factor	100	100	100	100	100	3.6	5.0	8.6	3.5 (3.2, 3.7)	NA
Prl	P06879	Prolactin	100	100	100	100	NA	3.3	3.5	9.9	2.4 (1.8, 2.9)	<1%
pSnca-129	O55042	Phophorylated Alpha-synuclein (S129)	93	100	100	100	41	18.0	19.6	27.8	7.9 (0.0, 9.2)	<1%
pTau-181	P10637	Phophorylated-Tau (T181)	100	100	100	87	100	13.5	4.2	18.7	9.1 (8.0, 9.5)	<1%
pTau-217	P10637	Phophorylated-Tau (T217)	100	100	100	100	100	14.3	10.3	21.6	10.8 (10.6, 11.4)	5.6% (pTau-231)
pTau-231	P10637	Phophorylated-Tau (T231)	100	100	100	100	100	7.6	4.6	11.1	7.6 (7.3, 8.0)	<1%
Rab10	P61027	Ras-related protein Rab-10	100	100	100	100	63	5.8	5.0	10.4	8.7 (8.4, 8.8)	NA
Rgma	Q6PCX7	Repulsive guidance molecule A	100	100	100	100	100	5.7	2.5	13.9	7.9 (7.2, 8.2)	<1%
S100a4	P07091	Protein S100-A4	100	100	100	100	100	4.2	6.5	8.6	0.0 (0.0, 0.1)	<1%
S100b	P50114	Protein S100-B	100	89	100	84	100	8.1	6.1	15.8	10.0 (9.9, 10.3)	<1%
Scg2	Q03517	Secretogranin-2	100	100	100	100	89	6.5	6.4	19.7	7.2 (6.8, 7.4)	<1%
Snap25	P60879	Synaptosomal-associated protein 25	100	89	97	74	96	5.3	4.1	11.9	11.6 (11.4, 11.8)	<1%
Snca	O55042	Alpha-synuclein	100	100	100	100	37	5.3	22.6	23.2	4.4 (4.0, 5.0)	2.2% (pSnca-129)
Tardbp	Q921F2	TAR DNA-binding protein 43	100	100	100	100	59	5.5	3.8	8.4	6.5 (6.3, 6.7)	<1%
Tau	P10637	Microtubule-associated protein tau	100	100	100	95	100	5.8	5.0	13.9	7.9 (7.7, 8.3)	<1%
Timp1	P12032	Metalloproteinase inhibitor 1	100	100	100	97	93	6.2	7.3	12.4	6.8 (6.6, 7.3)	<1%
Tnf	P06804	Tumor necrosis factor	100	100	100	100	44	5.3	4.7	7.6	8.1 (7.9, 8.4)	<1%
Tnfrsf11b	O08712	Tumor necrosis factor receptor superfamily member 11B	100	100	100	100	100	5.0	6.5	9.7	5.7 (5.4, 6.2)	<1%
Tnfrsf9	P20334	Tumor necrosis factor receptor superfamily member 9	100	100	100	100	59	4.3	4.9	6.9	3.3 (3.2, 3.7)	<1%
Tnfsf11	O35235	Tumor necrosis factor ligand superfamily member 11	100	100	100	100	59	8.1	5.7	10.3	0.0 (0.0, 0.0)	<1%
Tnfsf13b	Q9WU72	Tumor necrosis factor ligand superfamily member 13B	100	100	100	100	100	5.9	3.1	7.3	0.0 (0.0, 0.3)	NA
Tnfsf9	P41274	Tumor necrosis factor ligand superfamily member 9	100	100	100	100	100	5.1	5.6	11.4	7.1 (6.8, 7.3)	<1%
Trem2	Q99NH8	Triggering receptor expressed on myeloid cells 2	100	100	100	100	96	4.7	3.4	7.2	2.0 (1.7, 2.3)	<1%
Uchl1	Q9R0P9	Ubiquitin carboxyl-terminal hydrolase isozyme L1	100	100	100	95	100	4.8	7.3	15.1	10.8 (10.5, 11.2)	<1%
Vegfa	Q00731	Vascular endothelial growth factor A, long form	97	68	100	39	0	5.8	3.9	19.5	8.8 (8.7, 9.0)	<1%
Vegfb	P49766	Vascular endothelial growth factor B	100	100	100	100	93	5.0	4.4	19.7	6.5 (6.2, 6.8)	<1%
Vegfc	P97953	Vascular endothelial growth factor C	100	100	100	100	15	5.3	7.1	11.0	9.0 (8.9, 9.2)	1.0%
Vsnl1	P62761	Visinin-like protein 1	100	100	100	100	100	9.8	7.5	15.5	8.4 (8.1, 8.8)	<1%
Wnt16	Q9QYS1	Protein Wnt-16	100	100	100	100	100	3.4	4.4	6.3	7.8 (7.5, 7.8)	<1%

Note: Ifna5 exhibits 90% homology with Ifna1, potentially leading to 24.5% cross-reactivity; Ifna11 shares 89% homology with Ifna2, which could result in 4.2% cross-reactivity; ptau-231 may cause 5.6% cross-reactivity with ptau-217; and pSnca-129 could cause 2.2% cross-reactivity with Snca.

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