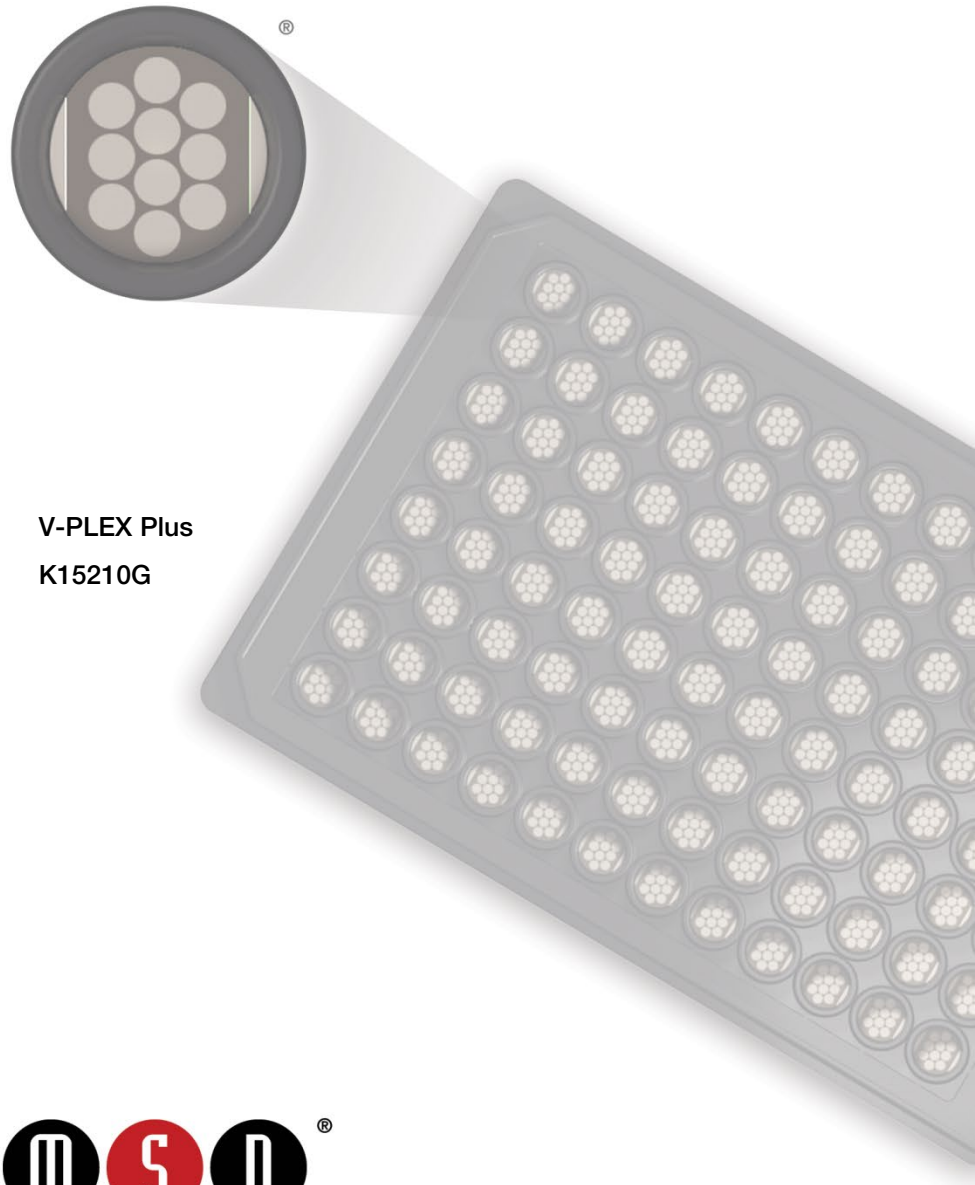
MSD® MULTI-SPOT Assay System

Neuroinflammation Panel 1 (human) Kits

CRP, Eotaxin, Eotaxin-3, FGF (basic), Flt-1, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PlGF, TARC, Tie-2, SAA, TNF-α, TNF-β, VCAM-1, VEGF-A, VEGF-C, VEGF-D

V-PLEX®



	V-PLEX®	V-PLEX Plus
Multiplex Kits	K15210D	K15210G

MSD®

Neuroinflammation Panel 1 (human) Kits

CRP, Eotaxin, Eotaxin-3, FGF (basic), VEGFR-1/Flt-1, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PlGF, TARC, Tie-2, SAA, TNF-α, TNF-β, VCAM-1, VEGF-A, VEGF-C, VEGF-D

For use with human cerebral spinal fluid (CSF).

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Introduction

MSD offers V-PLEX assays for customers who require unsurpassed performance and quality. V-PLEX products are developed under rigorous design control and are fully validated according to fit-for-purpose principles[1] following MSD's Quality Management System. They offer exceptional sensitivity, simple protocols, reproducible results, and lot-to-lot consistency. In addition to the analytical validation, the robustness of the assay protocol is assessed during development, along with the stability and robustness of the assay components and kits. V-PLEX assays are available in both single-assay and multiplex formats.

The V-PLEX assay menu is organized by panels. Grouping the assays into panels by species, analytical compatibility, clinical range, and expected use ensures optimal and consistent performance from each assay while still providing the benefits and efficiencies of multiplexing.

Mounting evidence suggests that neuroinflammation is not just a component of Alzheimer's disease (AD) but is also a potential driver of its progression.[2] This observation finds support in both basic research and clinical studies.[3-7] Clinical studies on AD samples from autopsied brains further strengthen the case for inflammation's role. Amyloid beta accumulation triggers a cascade of proinflammatory responses involving cytokines, chemokines, and other factors.[8,12] Recent research indicates that the foundation for ongoing and future therapeutic trials in this area lies in anti-inflammatory approaches aimed at modifying AD progression.[13,14]

MSD has developed a panel of 37 biomarkers to facilitate neuroinflammation studies. A large number of human CSF samples were analyzed from AD, Parkinson's disease, and other neurological disorders, as well as healthy samples. The assays in Neuroinflammation Panel 1 (human) were all able to detect their respective biomarkers in at least 10% of the samples tested.

Principle of the Assay

MSD assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Neuroinflammation Panel 1 (human) are sandwich immunoassays. The full panel consists of 5 plates (Proinflammatory Panel 1 Plate, Cytokine Panel 1 Plate, Chemokine Panel 1 Gen. B Plate, Angiogenesis Panel 1 Plate, and Vascular Injury Panel 2 Plate) each pre-coated with capture antibodies on 4 to 10 independent and well-defined spots as shown in the layout below. The assays are grouped based on optimal performance in a multiplex panel using multiple matrices. The analytes shown below have been detected in CSF.

The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD™ SULFO-TAG) throughout one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that creates the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample. V-PLEX assay kits have been validated according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J. W. Lee, et al.[1]

Proinflammatory Panel 1	Cytokine Panel 1	Chemokine Panel 1 Gen. B	Angiogenesis Panel 1	Vascular Injury Panel 2
IFN-γ	IL-1α	Eotaxin	VEGF-C	SAA
IL-1β	IL-5	MIP-1β	VEGF-D	CRP
IL-2	IL-7	Eotaxin-3	Tie-2	VCAM-1
IL-4	IL-12/IL-23p40	TARC	VEGFR-1/Flt-1	ICAM-1
IL-6	IL-15	IP-10	PlGF	
IL-8	IL-16	MIP-1α	FGF (basic)	
IL-10	IL-17A	MCP-1		
IL-13	TNF-β	MDC		
TNF-α	VEGF-A	MCP-4		

Figure 1. *Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.*

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional product-specific safety information is available in the applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Assay Protocol

The analytes in the Neuroinflammation Panel 1 (human) have been tested in CSF and the results are shown in this insert. Each group is provided with a panel-specific product insert containing the detailed assay protocol and additional results from validation testing in other matrices. For the detailed protocol, assay components, and data on precision, specificity, species-cross-reactivity, and stability, please refer to the panel-specific product insert. Summary protocols are also included in each product insert for your convenience.

All of the panels follow a general protocol similar to the one shown below. For the necessary details, please see the detailed protocol provided with each panel.

STEP 1: **Add Blocker A Solution** (This step is only necessary for Angiogenesis Panel 1 [human].)

- ❑ Add 150 µL/well of Blocker A Solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1 hour.

STEP 2: **Wash and Add Sample**

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 50 µL/well of the diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1–2 hours.

STEP 3: **Wash and Add Detection Antibody Solution**

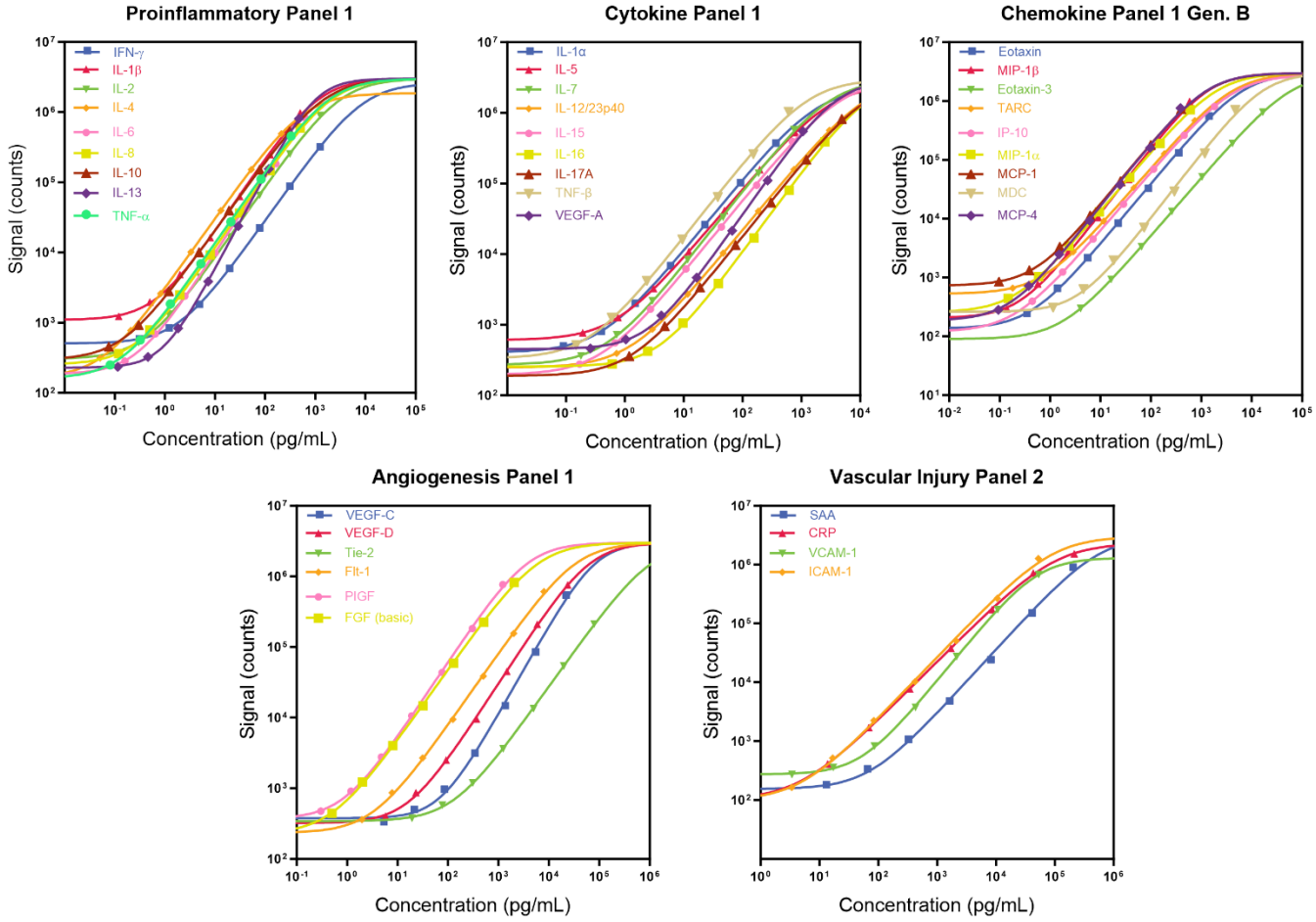
- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1–2 hours

STEP 4: **Wash and Read**

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL of Read Buffer (see Table 7 for more details on Read Buffer) to each well. Analyze the plate on the MSD instrument. Incubation in Read Buffer is not required before reading the plate.

Typical Data

Representative standard curves for each panel are presented below.



Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator).

The ULOQ is the highest concentration at which the CV of the calculated concentration is <20% (<25% for PlGF) and the recovery of each calibrator is within 80–120% of the known value (75% to 125% for TARC and MDC).

The LLOQ is the lowest concentration at which the CV of the calculated concentration is <20% (<25% for Flt-1, PlGF, and FGF (basic)), and the recovery of each calibrator is within 80–120% of the known value.

The quantitative range of the assay lies between the LLOQ and ULOQ.

The LLOQ and ULOQ are verified for each kit lot and the results are provided in the lot-specific COA that is included with each kit and available at www.mesoscale.com.

Table 1. LLOD, LLOQ, and ULOQ for each analyte in the Neuroinflammation Panel 1 (human) Kit

Panel	Biomarker	Median LLOD (pg/mL)	LLOD Range (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
Proinflammatory Panel 1	IFN-γ	0.37	0.21–0.62	1.76	938
	IL-1β	0.05	0.01–0.17	0.646	375
	IL-2	0.09	0.01–0.29	0.890	938
	IL-4	0.02	0.01–0.03	0.218	158
	IL-6	0.06	0.05–0.09	0.633	488
	IL-8	0.07	0.03–0.14	0.591	375
	IL-10	0.04	0.02–0.08	0.298	233
	IL-13	0.24	0.03–0.73	4.21	353
	TNF-α	0.04	0.01–0.13	0.690	248
Cytokine Panel 1	IL-1α	0.09	0.05–2.40	2.85	278
	IL-5	0.14	0.04–0.46	4.41	562
	IL-7	0.12	0.08–0.17	0.851	563
	IL-12/IL-23p40	0.33	0.25–0.42	1.32	2,250
	IL-15	0.15	0.09–0.25	0.774	525
	IL-16	2.83	0.88–9.33	19.1	1,870
	IL-17A	0.31	0.19–0.55	3.19	3,650
	TNF-β	0.08	0.04–0.17	0.465	458
	VEGF-A	1.12	0.55–6.06	7.70	562
Chemokine Panel 1 Gen. B	Eotaxin	0.20	0.13 - 0.61	2.14	910
	MIP-1β	0.09	0.04 - 0.25	1.20	390
	Eotaxin-3	1.44	0.89 - 2.21	11.1	11,100
	TARC	0.11	0.06 - 1.28	2.14	481
	IP-10	0.12	0.06 - 0.60	1.29	1,240
	MIP-1α	0.05	0.03 - 0.16	0.357	403
	MCP-1	0.16	0.03 - 2.60	1.09	247
	MDC	1.00	0.59 - 19.50	6.43	2,930
	MCP-4	0.05	0.03 - 0.50	0.518	234
Angiogenesis Panel 1	VEGF-C	10.5	7.11–17.5	146	17,500
	VEGF-D	4.36	1.83–70.6	67.1	18,800
	Tie-2	31.3	16.4–62.6	396	63,400
	VEGFR-1/Flt-1	0.90	0.65–1.21	10.0	6,410
	PlGF	0.21	0.04–1.42	1.50	800
	FGF (basic)	0.09	0.07–0.22	2.60	1,780
Vascular Injury Panel 2	SAA	10.9	1.07–35.5	54.0	138,000
	CRP	1.33	0.69–19.8	27.6	49,600
	VCAM-1	6.00	0.93–35.8	37.6	32,000
	ICAM-1	1.94	1.05–4.57	15.0	32,700

Dilution Linearity

To assess linearity, human CSF samples (N = 5–6) were spiked with recombinant calibrators and diluted before testing. The average percent recovery shown below is based on samples within the quantitative range of the assay.

$$\%\, recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 2. Analyte percent recovery at various dilutions in human CSF samples. Percent recovery at each dilution level was normalized to the dilution-adjusted concentration measured at the 2-fold dilution.

	IFN-γ		IL-1β		IL-2		IL-4		IL-6		IL-8	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
4	100	96–103	97	92–100	81	77–83	101	97–104	99	88–104	94	`82–98
8	94	89–100	93	88–96	73	71–75	100	96–106	95	84–98	92	88–98
16	93	87–101	89	83–94	67	65–71	98	93–104	89	80–93	85	76–94
32	90	85–94	89	87–93	67	63–72	97	93–99	85	79–89	89	85–96
64	93	87–99	89	85–94	65	63–70	98	92–106	88	82–92	84	74–94

	IL-10		IL-13		TNF-α		IL-1α		IL-5		IL-7	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
4	98	94–102	88	85–91	91	89–94	106	102–111	110	98–127	107	100–119
8	96	95–98	81	77–84	85	83–88	110	103–114	115	99–139	100	90–116
16	92	90–95	73	70–77	80	77–85	108	100–113	115	97–140	98	95–100
32	93	90–95	71	67–76	82	78–87	115	104–129	117	106–133	94	86–104
64	92	88–97	70	65–76	80	75–86	116	113–119	117	104–131	102	94–108

	IL-12/IL-23p40		IL-15		IL-16		IL-17A		TNF-β		VEGF-A	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
4	109	101–117	91	82–101	99	92–114	118	111–128	114	108–120	102	100–107
8	107	97–121	103	87–133	99	90–113	113	99–124	121	115–136	105	97–113
16	105	96–122	107	87–142	96	88–110	111	107–123	123	112–142	105	92–116
32	106	94–118	118	93–160	96	92–103	108	94–119	129	118–151	117	96–137
64	111	102–124	119	91–157	98	89–118	112	102–118	134	118–159	126	99–153

Table 3. Analyte percent recovery at various dilutions in human CSF samples. Percent recovery at each dilution level was normalized to the dilution-adjusted concentration measured at the 4-fold dilution.

	Eotaxin		MIP-1β		Eotaxin-3		TARC		IP-10		MIP-1α	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
4	100	N/A	100	N/A	100	N/A	100	N/A	100	N/A	100	N/A
8	99	90–110	96	86–101	97	91–105	96	87–108	113	95–125	92	90–96
16	111	93–127	91	85–96	97	88–106	105	91–118	126	109–137	91	87–95
32	111	96–128	91	87–94	95	88–103	96	83–111	133	111–149	88	84–92

NA = not applicable

	MCP-1		MDC		MCP-4	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
4	100	N/A	100	N/A	100	N/A
8	95	87–100	101	96–104	105	99–109
16	97	88–102	112	107–120	113	105–120
32	94	87–99	116	108–122	118	110–125

NA = not applicable

Table 4. Human CSF samples (N = 8) were spiked with recombinant calibrators and diluted 5-fold, 10-fold, 50-fold, and 100-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted concentration measured at the 5-fold dilution.

	SAA		CRP		VCAM-1		ICAM-1	
Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
10	98	89–106	106	99–118	107	99–115	102	91–113
50	ND	—	107	96–115	124	104–136	103	91–116
100	ND	—	110	98–116	135	114–153	102	87–118

ND = not detected; dash (—) = not applicable

Spike Recovery

Spike recovery measurements throughout the quantitative range of the assays were evaluated. Human CSF samples were obtained from a commercial source. Samples (N = 5–6) were spiked with calibrators at 3 levels (high, mid, and low) and then diluted either 2-fold (Proinflammatory and Cytokine panels) or 4-fold (Chemokine Gen. B panel). The average percent recovery for each sample type is reported along with %CV and percent recovery range.

$$\%\ recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 5. Spike recovery measurements of CSF samples in the Proinflammatory, Cytokine, and Chemokine Gen. B panels of the Neuroinflammation Panel 1 (human) Kit

Panel	Biomarker	Average % Recovery	%CV	% Recovery Range
Proinflammatory Panel 1	IFN-γ	65	3.9	59–69
	IL-1β	93	7.7	83–101
	IL-2	121	3.2	117–125
	IL-4	92	7.3	85–103
	IL-6	92	6.7	85–102
	IL-8	100	2.9	95–102
	IL-10	88	2.8	83–91
	IL-13	112	2.6	110–115
	TNF-α	111	3.3	107–116
Cytokine Panel 1	IL-1α	77	24.0	35–96
	IL-5	87	2.9	84–90
	IL-7	87	2.1	84–90
	IL-12/IL-23p40	99	5.9	93–107
	IL-15	90	3.3	85–93
	IL-16	85	6.3	78–94
	IL-17A	98	5.3	90–104
	TNF-β	105	7.7	93–113
	VEGF-A	121	15.9	109–148
Chemokine Panel 1 Gen. B	Eotaxin	100	5.6	92–110
	MIP-1β	101	6.2	90–111
	Eotaxin-3	86	14.0	60–98
	TARC	122	10.6	103–159
	IP-10	75	8.5	67–83
	MIP-1α	118	3.9	110–126
	MCP-1	101	N/A	N/A
	MDC	95	6.7	84–104
	MCP-4	94	5.3	86–103

N/A = not applicable

Tested Samples

Combinations of pooled and individual, normal, and diseased samples from commercially available human CSF were measured. Results for each sample set are displayed below. Concentrations are corrected for sample dilution. The median and range are calculated from samples with concentrations at or above the LLOD. The percentage detected is the percentage of samples with concentrations at or above the LLOD.

Table 6. *Results for each sample set tested in the Neuroinflammation Panel 1 (human) Kit*

Panel	Biomarker	N	Median (pg/mL)	Range (pg/mL)	% Detected
Proinflammatory Panel 1	IFN-γ	121	0.99	0.33–554	50
	IL-1β	121	0.40	0.20–97.2	44
	IL-2	121	0.30	0.15–11.6	44
	IL-4	121	0.06	0.03–1.80	15
	IL-6	121	1.39	0.52–4,100	74
	IL-8	121	56.1	22.3–4,420	98
	IL-10	121	0.15	0.05–76.9	55
	IL-13	121	2.68	1.16–23.1	40
	TNF-α	121	0.18	0.05–59.5	60
Cytokine Panel 1	IL-1α	121	0.37	0.07–16.3	61
	IL-5	121	0.51	0.16–4.80	66
	IL-7	121	1.08	0.33–6.42	88
	IL-12/IL-23p40	121	4.39	0.70–795	93
	IL-15	121	2.55	0.87–14.5	98
	IL-16	121	7.89	2.68–687	67
	IL-17A	121	1.68	0.34–74.4	16
	TNF-β	121	0.16	0.08–3.25	12
	VEGF-A	121	2.74	0.96–51.0	82
Chemokine Panel 1 Gen. B	Eotaxin	10	2.65	0.96–8.15	100
	MIP-1β	10	8.44	3.86–20.5	100
	Eotaxin-3	10	9.85	9.32–10.4	20
	TARC	10	2.70	2.03–112	90
	IP-10	10	255	47.8–9,990	100
	MIP-1α	10	2.60	0.64–14.1	100
	MCP-1	10	436	115–891	100
	MDC	10	6.85	3.72–42.3	80
	MCP-4	10	0.50	0.22–5.25	100
Angiogenesis Panel 1	VEGF-C	11	31.9	15.5–44.0	73
	VEGF-D	8	52.5	24.4–140	100
	Tie-2	8	70.7	25.3–253	100
	VEGFR-1/Flt-1	8	38.8	30.2–172	100
	PlGF	8	32.9	6.83–71.1	100
	FGF (basic)	8	0.45	0.29–1.11	100
Vascular Injury Panel 2	SAA	21	11,400	854–168,000	90
	CRP	21	30,900	1,170–1,030,000	95
	VCAM-1	21	10,400	4,780–79,500	100
	ICAM-1	21	4,300	1,690–46,400	100

Over half the assays in the panel detected analyte in at least 75% of samples measured, and all assays in the panel detected analyte in at least 10% of samples. The data shown in Table 6 is represented graphically below, with analytes grouped by abundance. The cytokine assays in the panel were used to test both diseased (red lines) and normal (blue line) samples (N = 121). Only normal samples (N = 2–21) were tested with Eotaxin, Eotaxin-3, FGF (basic), MCP-4, MDC, MIP-1α, MIP-1β, CRP, ICAM-1, IP-10, MCP-1, PlGF, SAA, sFlt-1, Tie-2, VCAM-1, VEGF-C, and VEGF-D.

At the recommended dilutions, endogenous levels of cytokines were readily detected and quantified in CSF obtained from a wide range of samples. Importantly, the assays have sufficient dynamic range to simultaneously measure endogenous levels of cytokines in a general population as well as abnormal levels associated with disease. The dynamic range and precision exhibited by these multiplex assays may improve biomarker discovery when comparing appropriately matched cases and controls for AD, Parkinson's disease, or other neurological diseases. Overall, these results demonstrate the utility of the Neuroinflammation Panel 1 (human) as a multiplex tool for measuring a broad range of cytokines and other biomarkers in CSF.

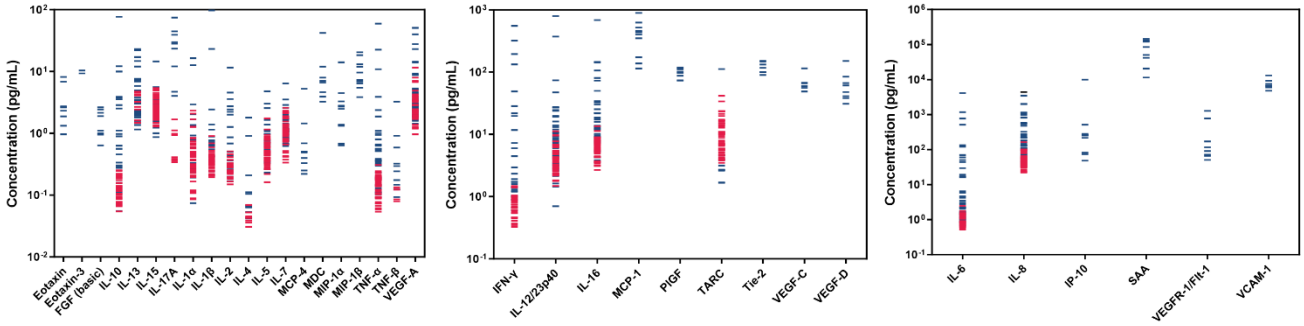


Figure 2. Dynamic range for each sample set tested in the Neuroinflammation Panel 1 (human) Kit

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Appendix A: Neuroinflammation Panel 1 (human) Protocol Comparison

The assay protocol for each panel is summarized below. For the detailed protocols, please refer to the panel-specific product inserts available at www.mesoscale.com.

Table 7. Assay protocol details for each panel in the Neuroinflammation Panel 1 (human) Kit

Details	Proinflammatory Panel 1	Cytokine Panel 1	Chemokine Panel 1 Gen. B	Angiogenesis Panel 1	Vascular Injury Panel 2
Wash Plate Before Use	No*	No*	No*	No	Yes
Blocking Step	No	No	No	Blocker A, 1 hour	No
Sample/Calibrator Diluent	Diluent 2	Diluent 43	Diluent 57	Diluent 7	Diluent 101
Calibrator Storage	Lyophilized, 2–8 ºC	Lyophilized, 2–8 ºC	Lyophilized, 2–8 ºC	≤–70 ºC	≤–70 ºC
Calibrator Curve Dilution Series	4-fold	4-fold	4-fold	4-fold	5-fold
Control Storage	Lyophilized, 2–8 ºC	Lyophilized, 2–8 ºC	Lyophilized, 2–8 ºC	≤–70 ºC	≤–70 ºC
Control Dilution	1:2	1:2	1:4	1:2	No
Serum/Plasma Sample Dilution	1:2	1:2	1:4	1:2	1:1000
CSF Sample Dilution	1:2	1:2	1:4	1:2	1:5
Calibrator/Sample/Control Volume/well	50 µL	50 µL	50 µL	50 µL	25 µL
Calibrator/Sample/Control Incubation	2 hours	2 hours	2 hours	2 hours	2 hours
Antibody Diluent	Diluent 3	Diluent 3	Diluent 3	Diluent 11	Diluent 101
Detection Antibody Volume	25 µL	25 µL	25 µL	25 µL	25 µL
Detection Antibody Incubation	2 hours	2 hours	2 hours	2 hours	1 hour
Read Buffer	MSD Read Buffer T (4X)	MSD Read Buffer T (4X)	MSD GOLD Read Buffer B	MSD Read Buffer T (4X)	MSD Read Buffer T (4X)
Working Read Buffer Concentration	2X	2X	Use as is	2X	1X

*Washing the plate before sample addition is an optional step that may provide greater uniformity of results for certain assays. Analytical parameters, including limits of quantification, recovery of controls, and sample quantification, are not affected by washing the plate before sample addition.