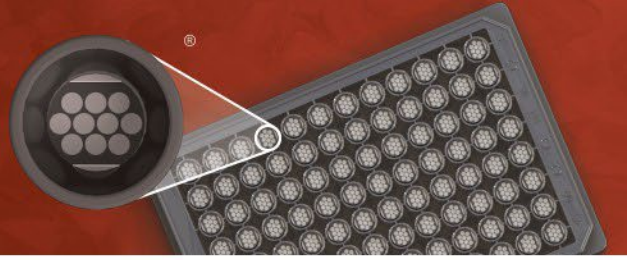
V-PLEX®

Cytolytic Panel 1 (human) Kit

Datasheet



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Ordering Information

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Products Covered

Multiplex Kit	V-PLEX	V-PLEX Plus*
V-PLEX® Cytolytic Panel 1 (human) Kit	K15741D	K15741G
Individual Assay Kits		
Human GM-CSF V2	K151AVAD	K151AVAG
Human Granzyme B	K151AVBD	K151AVBG
Human IFN-γ V2	K151AVCD	K151AVCG
Human IL-2	K151QQD**	K151QQG**
Human IL-6 V2	K151AVED	K151AVEG
Human Perforin	K151AVFD	K151AVFG
Human TNF-α V2	K151AVGD	K151AVGG

*V-PLEX Plus kits contain 3 control reagents in addition to the components in the V-PLEX kit.
** Performance represented in this product is based on multiplex kit.
Product insert and certificates of analysis are available at https://www.mesoscale.com/en/products_and_services/assay_kits.
For a complete list of products, please visit our website at www.mesoscale.com.

Introduction

The MESO SCALE DISCOVERY® (MSD) V-PLEX Cytolytic Panel 1 (human) Kit measures Granulocyte-macrophage colony-stimulating factor (GM-CSF), Granzyme B, Interferon gamma (IFN-γ), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Perforin, and Tumor necrosis factor alpha (TNF-α). The markers of the V-PLEX Cytolytic Panel 1 are curated to capture key features of immune cell activation and cytolytic processes, particularly from cytotoxic T cells and Natural Killer (NK) cells. These factors are produced following immune recognition and engagement of infected, stressed, or malignant target cells in both *in vitro* model systems and *in vivo* biological samples. By combining effector cytokines with cytolytic mediators such as Granzyme B and Perforin, the panel enables simultaneous assessment of immune activation and target cell killing mechanisms, supporting comprehensive characterization of cytolytic immune responses within a single assay kit.

This multiplex kit is validated using a simplified two-step immunoassay protocol that delivers results in under 3 hours.

V-PLEX Characteristics

V-PLEX biomarker products typically provide sensitivity in the pg/mL or better range, enabling detection across a wide range of analyte expression levels.

V-PLEX assays are available as singleplex kits or multiplex panels, which can be customized to create subsets ideal for specific research needs. Grouping the assays into panels by species, analytical compatibility, expression levels, and expected use ensures optimal and consistent performance.

Available reference standards and controls guarantee that every kit provides consistent performance and delivers results that are compatible with international standards. The inclusion of pre-coated V-PLEX plates ensures a simple and reliable workflow that limits sources of variability.

Analytical Validation Data

Analytical validation of the Cytolytic Panel 1 (human) Kit was performed using three kit lots. The Methodical Mind Enterprise™ analysis software was used to convert electrochemiluminescence signal to analyte concentrations. Validation data is presented in the following sections.

Calibration Curves

For each plate, a calibration curve was generated by fitting signals from 7 calibrators and a blank to a 4-parameter logistic (4PL) model with $1/Y^2$ weighting. Calibrators were prepared by serial dilution of the recommended top calibrator concentration, with the range designed to ensure the lowest calibrator fell below the expected LLOQ to improve accuracy at low analyte concentrations. Representative calibration curves from one kit lot are presented in Figure 1.

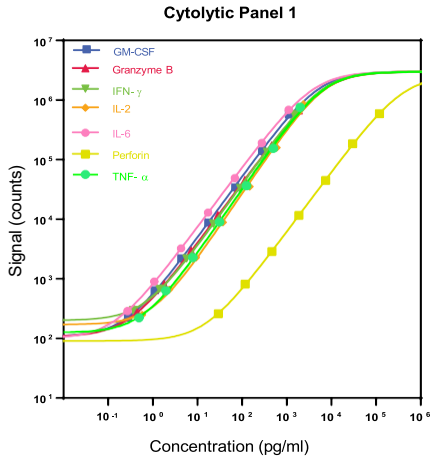

Figure 1. *Representative calibration curves for assays in the V-PLEX Cytolytic Panel 1 (human) Kit.*

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the mean signal 2.5 standard deviations above the background (zero calibrator). The LLOD is calculated across multiple plates per lot; the median and range for a representative kit lot are reported in Table 1.

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

Analyte	Median LLOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)	Dynamic Range (pg/mL)
GM-CSF	0.15	0.676	910	0.15 – 910
Granzyme B	0.17	0.657	1,300	0.17 – 1,300
IFN-γ	0.19	0.748	1,100	0.19 – 1,100
IL-2	0.43	1.76	1,310	0.43 – 1,310
IL-6	0.10	0.436	780	0.10 – 780
Perforin	11	53.8	84,500	11 – 84,500
TNF-α	0.25	0.898	1,360	0.25 – 1,360

The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) are established by measuring independent samples near the bottom and top of the calibration range, respectively, using the classical method. Each limit is set at the concentration where the %CV of back-calculated concentration values is less than 20% and concentration recovery is within 80–120% of the nominal value. Both limits were established across multiple kit lots, are verified for each kit lot, and are reported on the lot-specific Certificate of Analysis available at www.mesoscale.com.

The dynamic range of each assay spans from the LLOD to the ULOQ, typically covering 3–5 log units. The quantitative range, defined as the interval between the LLOQ and ULOQ, typically spans 3–4 log units.

Precision

Independent quality control samples at three different concentrations for each analyte were run on every plate to assess performance of the kit. Precision was based on 48 runs performed by 4 operators. Each control was tested in triplicate over multiple days (Table 2).

Table 2. Intra-run and inter-run %CVs for each analyte in the Cytolytic Panel 1 (human) Kit

Analyte	Control	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV	Inter-lot %CV
GM-CSF	Control 1	580	3.8	4.4	4.1
	Control 2	51	2.3	5.8	3.0
	Control 3	4	3.1	11	6.1
Granzyme B	Control 1	760	4.5	5.4	2.1
	Control 2	55	2.3	6.8	5.1
	Control 3	4	3.1	12	6.8
IFN-γ	Control 1	680	3.4	4.9	1.7
	Control 2	53	2.6	6.5	3.5
	Control 3	4	4.3	10	5.3
IL-2	Control 1	1,400	3.9	5.4	6.4
	Control 2	140	2.6	6.2	9.0
	Control 3	15	3.8	11	3.7
IL-6	Control 1	470	5.1	5.7	3.8
	Control 2	35	2.8	6.3	5.3
	Control 3	3	3.2	11	4.0
Perforin	Control 1	62,000	7.6	7.0	5.9
	Control 2	4,700	4.2	6.6	6.4
	Control 3	350	5.4	12	7.5
TNF-α	Control 1	760	4.1	5.4	5.2
	Control 2	68	3.2	7.0	3.5
	Control 3	5	3.7	11	3.3

The intra-run concentration %CV was computed using the triplicates within a plate. The inter-run concentration %CV was computed using all the replicates across the 48 runs. The inter-lot concentration %CV was computed from the average concentration measured within each kit lot. The results show that the average intra-run %CV and inter-lot %CV are all less than 10%. The inter-run %CV is typically less than 10%, well below the acceptance specification of 20%.

Accuracy and precision of controls are measured as part of lot release for each kit lot. Results are reported on the lot-specific Certificate of Analysis available at www.mesoscale.com and meet typical acceptance criteria of %CV less than 20% and recovery between 80–120%.

Sample Ranges

Serum, EDTA plasma, heparin plasma, and citrate plasma were commercially sourced. Serum and plasma samples were diluted 2-fold. Results for each sample type are displayed in Table 3. 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 3. Human samples tested using the Cytolytic Panel 1 (human) Kit

Analyte	Statistic	Serum (N=10)	EDTA Plasma (N=10)	Heparin Plasma (N=10)	Citrate Plasma (N=10)
GM-CSF	Median (pg/mL)	0.39	ND	ND	ND
	Range (pg/mL)	0.39	-	-	-
	% Detected	10	0	0	0
Granzyme B	Median (pg/mL)	1.3	1.7	1.2	1.5
	Range (pg/mL)	0.94-4.3	0.82-3.2	0.60-2.4	0.78-3.2
	% Detected	100	100	100	100
IFN-γ	Median (pg/mL)	0.87	0.98	0.88	1.0
	Range (pg/mL)	0.59-19	0.64-20	0.65-19	0.60-17
	% Detected	50	40	50	30
IL-2	Median (pg/mL)	ND	ND	0.89	ND
	Range (pg/mL)	-	-	0.89	-
	% Detected	0	0	10	0
IL-6	Median (pg/mL)	1.4	1.0	2.0	1.0
	Range (pg/mL)	0.72-4.3	0.60-4.6	0.58-9.1	0.63-4.0
	% Detected	100	100	100	100
Perforin	Median (pg/mL)	15000	20000	13000	13000
	Range (pg/mL)	6300-19000	9200-27000	6200-18000	6300-17000
	% Detected	100	100	100	100
TNF-α	Median (pg/mL)	9.9	8.6	8.0	7.4
	Range (pg/mL)	6.7-110	5.7-110	5.2-100	4.6-98
	% Detected	100	100	100	100

ND = Not Detectable

Dilution Linearity

To assess linearity, commercially sourced normal human samples were spiked with calibrator before testing as indicated. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration. The average percent recovery shown below (Table 4) is based on samples within the quantitative range of the assay.

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

Table 4a. Percent recovery at various dilutions in different sample types.

Analyte	Fold Dil.	Serum (N=8)		EDTA Plasma (N=8)		Heparin Plasma (N=8)		Citrate Plasma (N=8)	
		Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range
GM-CSF	2	100	-	100	-	100	-	100	-
	4	111	102-119	106	101-113	108	98-114	109	105-115
	8	104	90-111	103	97-111	102	96-109	104	99-111
	16	102	92-111	101	94-112	101	90-109	103	94-113
Granzyme B	2	100	-	100	-	100	-	100	-
	4	116	106-127	112	105-121	112	103-119	112	107-121
	8	112	100-124	112	103-125	109	101-117	111	104-121
	16	110	97-127	108	101-115	106	98-116	109	99-119
IFN-γ	2	100	-	100	-	100	-	100	-
	4	108	103-114	104	101-108	107	99-113	104	100-107
	8	105	100-114	105	100-109	108	99-113	104	98-108
	16	102	95-108	100	93-105	110	102-118	101	97-107
IL-2	2	100	-	100	-	100	-	100	-
	4	99	91-108	95	91-100	97	89-104	99	95-110
	8	89	78-100	89	83-93	87	81-93	90	83-98
	16	85	72-98	85	76-96	84	76-94	88	77-102
IL-6	2	100	-	100	-	100	-	100	-
	4	117	109-125	111	108-115	115	103-124	114	108-123
	8	112	104-123	110	108-116	114	109-124	113	105-125
	16	114	104-128	107	102-115	117	110-125	114	108-123
Perforin	2	100	-	100	-	100	-	100	-
	4	120	106-128	113	103-122	120	105-131	110	99-121
	8	117	96-136	88	78-100	117	101-135	110	98-127
	16	122	94-154	81	70-91	123	95-139	110	96-128
TNF-α	2	100	-	100	-	100	-	100	-
	4	105	96-115	102	92-108	105	97-114	103	94-112
	8	100	84-125	104	90-119	103	87-131	100	86-124
	16	100	80-142	101	87-130	102	83-141	97	79-129

Fold Dil. = Fold Dilution, Av.% Rec. = Average % Recovery, %Rec. Range = % Recovery Range, ND = Not Detectable

Table 4b. Percent recovery at various dilutions in different cell culture media types.

Analyte	DMEM + Glutamax		Neurobasal Medium		RPMI 1640	
	Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range
GM-CSF	97	92-101	93	84-97	104	101-106
Granzyme B	101	91-114	99	87-107	109	105-111
IFN-γ	95	89-100	90	83-93	97	94-100
IL-2	81	74-88	78	66-86	90	84-96
IL-6	98	94-103	97	87-104	108	104-110
Perforin	97	90-105	107	95-122	108	103-115
TNF-α	83	78-86	82	73-88	86	83-91

Parallelism

To assess linearity, commercially sourced samples were diluted as indicated before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (Table 5).

Table 5. Percent recovery at various dilutions in different sample types.

Analyte	Fold Dil.	Serum (N=8)		EDTA Plasma (N=8)		Heparin Plasma (N=8)		Citrate Plasma (N=8)	
		Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range	Av.% Rec.	%Rec. Range
GM-CSF	2	ND	-	ND	---	ND	---	ND	---
	4	ND	ND	ND	ND	ND	ND	ND	ND
	8	ND	ND	ND	ND	ND	ND	ND	ND
	16	ND	ND	ND	ND	ND	ND	ND	ND
Granzyme B	2	100	-	100	-	100	-	100	-
	4	109	109 - 109	117	117 - 117	108	104 - 112	115	115 - 115
	8	125	125 - 125	ND	ND	ND	ND	ND	ND
	16	ND	ND	ND	ND	ND	ND	ND	ND
IFN-γ	2	100	-	100	-	100	-	100	-
	4	98	98 - 98	103	103 - 103	106	106 - 106	108	108 - 108
	8	97	97 - 97	107	107 - 107	106	106 - 106	109	109 - 109
	16	87	87 - 87	108	108 - 108	110	110 - 110	101	101 - 101
IL-2	2	ND	---	ND	---	ND	---	ND	---
	4	ND	ND	ND	ND	ND	ND	ND	ND
	8	ND	ND	ND	ND	ND	ND	ND	ND
	16	ND	ND	ND	ND	ND	ND	ND	ND
IL-6	2	100	-	100	-	100	-	100	-
	4	115	108 - 127	124	110 - 133	114	106 - 122	125	119 - 132
	8	117	117 - 117	138	138 - 138	115	110 - 122	127	127 - 127
	16	ND	ND	ND	ND	117	113 - 120	ND	ND
Perforin	2	100	-	100	-	100	-	100	-
	4	124	107 - 135	121	113 - 128	117	109 - 125	117	107 - 128
	8	129	112 - 138	95	82 - 112	122	112 - 137	122	111 - 136
	16	137	114 - 153	89	74 - 100	129	105 - 151	131	117 - 152
TNF-α	2	100	-	100	-	100	-	100	-
	4	107	98 - 115	110	101 - 118	106	97 - 112	111	101 - 117
	8	112	98 - 133	118	102 - 130	114	94 - 129	121	111 - 131
	16	148	148 - 148	132	114 - 151	130	119 - 145	148	148 - 148

Fold Dil. = Fold Dilution, Av.% Rec. = Average % Recovery, %Rec. Range = % Recovery Range, ND = Not Detectable

Spike Recovery

Spike recovery measurements of different sample types were evaluated throughout the quantitative range of the assays (Table 6). Tested matrices were commercially sourced normal human samples and cell culture media. Samples were spiked with recombinant analyte protein at three levels (high, mid, and low) and then diluted 2-fold. 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 6a. Spike recovery measurements of sample matrices evaluated in the V-PLEX Cytolytic Panel 1 (human) Kit

Analyte	Serum (N=8)			EDTA Plasma (N=8)			Heparin Plasma (N=8)			Citrate Plasma (N=8)		
	Av.% Rec.	%CV	%Rec. Range	Av.% Rec.	%CV	%Rec. Range	Av.% Rec.	%CV	%Rec. Range	Av.% Rec.	%CV	%Rec. Range
GM-CSF	111	2.6	98 – 129	107	1.6	97 – 114	107	2.1	95 – 118	105	2.4	94 – 115
Granzyme B	104	2.3	90 – 125	97	2.0	78 – 109	102	1.7	90 – 112	102	1.9	90 – 113
IFN-γ	115	2.6	106 – 131	112	1.9	98 – 127	112	2.1	97 – 123	113	1.9	105 – 122
IL-2	125	3.4	95 – 149	121	2.6	99 – 140	122	2.7	101 – 140	123	2.7	99 – 148
IL-6	101	2.0	85 – 119	96	3.9	78 – 109	94	1.9	82 – 103	98	2.9	90 – 109
Perforin	103	3.4	77 – 131	132	4.5	115 – 163	98	5.1	80 – 113	101	6.1	74 – 116
TNF-α	104	3.8	65 – 119	98	3.7	67 – 114	105	2.8	63 – 124	103	3.0	65 – 122

Av.% Rec. = Average % Recovery, %Rec. Range = % Recovery Range

Table 6b. Spike recovery measurements of cell culture media evaluated in the V-PLEX Cytolytic Panel 1 (human) Kit

Analyte	DMEM + Glutamax			Neurobasal medium			RPMI		
	Av.% Rec.	%CV	%Rec. Range	Av.% Rec.	%CV	%Rec. Range	Av.% Rec.	%CV	%Rec. Range
GM-CSF	118	6.0	108 - 129	121	6.1	109 - 131	113	8.8	103 - 122
Granzyme B	114	6.6	100 - 124	114	9.3	97 - 127	113	11.0	100 - 125
IFN-γ	113	2.7	111 - 120	119	5.7	113 - 130	114	5.9	108 - 121
IL-2	140	4.8	128 - 150	142	8.1	127 - 159	135	8.0	124 - 146
IL-6	115	5.7	107 - 128	115	7.5	101 - 126	108	7.0	100 - 115
Perforin	119	4.9	107 - 127	119	5.8	107 - 126	116	5.2	109 - 121
TNF-α	125	3.5	119 - 132	131	7.0	123 - 143	123	5.7	119 - 131

Specificity

To assess specificity, each assay in the panel was tested individually. Nonspecific binding was less than 1% for all assays in the kit. Non-specificity reported in the COA for this panel is measured using blended calibrators and individual detection antibodies.

$$\%\, nonspecificity = \frac{nonspecific\ signal}{specific\ signal} \times 100$$

Assay Components

Calibrators

Table 7. Expression systems used for the calibrators in the Cytolytic Panel 1 (human) Kit.

Calibrator	Source
GM-CSF	*E. coli*
Granzyme B	NS0
IFN-γ	*E. coli*
IL-2	*E. coli*
IL-6	*E. coli*
Perforin	HEK293
TNF-α	*E. coli*

Calibration

All the assays in the panel are calibrated against a reference calibrator generated at MSD.

MSD reference calibrators for the following analytes were evaluated against the NIBSC/WHO International Standards; the ratios of International Units of biological activity per mL (IU/mL) of NIBSC/WHO standard relative to pg/mL of MSD calibrator are shown in the table below. To convert MSD concentrations to biological activity relative to the NIBSC/WHO International Standard, multiply the MSD concentration by the ratio provided.

Table 8. NIBSC to MSD conversion for analytes in the Cytolytic Panel 1 (human) Kit.

Analyte	Standard	NIBSC pg/mL : MSD pg/mL	NIBSC IU/mL : MSD pg/mL
GM-CSF	88/646	0.344	0.00344
IFN-γ	87/586	1.87	0.0373
IL-2	86/500	1.01	0.0139
IL-6	21/308	1.23	0.0529
TNF-α	12/154	1.45	0.0623

Antibodies

Table 9. Source of the capture and detection antibodies in the Cytolytic Panel 1 (human) Kit.

Antigen	Capture	Detection	Assay Generation
GM-CSF	Recombinant Monoclonal	Recombinant Monoclonal	B
Granzyme B	Recombinant Monoclonal	Recombinant Monoclonal	A
IFN-γ	Recombinant Monoclonal	Recombinant Monoclonal	D
IL-2	Recombinant Monoclonal	Recombinant Monoclonal	B
IL-6	Recombinant Monoclonal	Recombinant Monoclonal	D
Perforin	Recombinant Monoclonal	Recombinant Monoclonal	A
TNF-α	Recombinant Monoclonal	Recombinant Monoclonal	C