Special Order Human Biomarker Group 1

V-PLEX® assays: bFGF, Eotaxin, Eotaxin-3, Flt-1, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA*), IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IP-10, PlGF, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, Tie-2, TNF-α, TNF-β, VEGF-A, VEGF-C, VEGF-D

*High Abundance (This assay quantitates high levels of IL-8.)

Other MSD catalog human assays: BDNF, β-NGF, CA-125, Calbindin, CTACK, EGF, EPO, E-Selectin, FAS Ligand, Fractalkine, G-CSF, GRO-α, I-309, ICAM-3, IFN-α2a, IL-6R, IL-17B, IL-17D, IL-18, I-TAC, MCP-2, MCP-3, M-CSF, MIG, MIP-3α, MIP-3β, MIP-5, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, NT-ProBNP, Osteoactivin, Osteonectin, OPGN, Osteopontin, P-Selectin, RANTES, SDF-1α, TNF-RI, TNF-RII, TPO, TRAIL, YKL-40

Special Order Kits	5-Plate Kit	25-Plate Kit
Catalog #	K151A4I-2	K151A4I-4

This protocol may be used for combinations of up to 10 of the assays above per plate.

MSD Cytokine Assays

Special Order Human Biomarker Group 1 Kits

V-PLEX assays: bFGF, Eotaxin, Eotaxin-3, Flt-1, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA*), IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IP-10, PlGF, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, Tie-2, TNF-α, TNF-β, VEGF-A, VEGF-C, VEGF-D

*High Abundance (This assay quantitates high levels of IL-8.)

Other MSD catalog human assays: BDNF, β-NGF, CA-125, Calbindin, CTACK, EGF, EPO, E-Selectin, FAS Ligand, Fractalkine, G-CSF, GRO-α, I-309, ICAM-3, IFN-α2a, IL-6R, IL-17B, IL-17D, IL-18, I-TAC, MCP-2, MCP-3, M-CSF, MIG, MIP-3α, MIP-3β, MIP-5, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, NT-ProBNP, Osteoactivin, Osteonectin, OPGN, Osteopontin, P-Selectin, RANTES, SDF-1α, TNF-RI, TNF-RII, TPO, TRAIL, YKL-40

For use with cell culture supernatants, serum, plasma, and urine.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

Special order kits allow customers to combine validated V-PLEX assays in new configurations or mix V-PLEX assays with selected assays from MSD's standard menu. This allows customers to create unique multiplex assays with greater confidence in performance. The assays listed below have been tested by MSD for analytical compatibility, and data on analytical performance, calibration curves, reproducibility, and specificity are provided in the certificate of analysis included with each kit. Special order applications may be unique; therefore, users should test abundance levels in their specific samples and matrices to determine the optimum combination of assays.

Assays that can be combined into a Special Order Human Biomarker Kit (Group 1) are:

MSD Human V-PLEX Assays

Proinflammatory Panel 1: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α
Chemokine Panel 1: Eotaxin, MIP-1β, Eotaxin-3, TARC, IP-10, MIP-1α, IL-8 (HA*), MCP-1, MDC, MCP-4
Cytokine Panel 1: GM-CSF, IL-1α, IL-5, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, VEGF-A
Angiogenesis Panel 1: VEGF-A, VEGF-C, VEGF-D, Tie-2, Flt-1, bFGF, PlGF

*High Abundance (This assay quantitates high levels of IL-8.)

Other Analytically Compatible MSD Human Assays

BDNF, β-NGF, CA-125, Calbindin, CTACK, EGF, EPO, E-selectin, FAS ligand, Fractalkine, G-CSF, GRO-α, I-309, ICAM-3, IFN-α2a, IL-6R, IL-17B, IL-17D, IL-18, I-TAC, MCP-2, MCP-3, M-CSF, MIG, MIP-3α, MIP-3β, MIP-5, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, NT-ProBNP, Osteoactivin, Osteonectin, OPGN, Osteopontin, P-selectin, RANTES, SDF-1α, TNF-RI, TNF-RII, TPO, TRAIL, YKL-40.

The kit contains plates pre-coated with specific capture antibodies. Other reagents (calibrators and detection antibodies) are provided based on the assays selected. Custom V-PLEX reagents are the same as those provided in the pre-configured V-PLEX kits. V-PLEX calibrators are lyophilized or frozen multi-analyte calibrators that have been referenced to an internal MSD standard. Based on the combination of biomarkers chosen from the human V-PLEX panels, 1 to 4 multi-analyte calibrators may be supplied. Calibrators for the other assays are supplied from the same bulk materials that are used for individual MSD assays. These individual calibrators are supplied at 50 µg/mL so that there will be ample material for blending with the multi-analyte V-PLEX calibrator(s). V-PLEX detection antibodies have been developed to minimize signal variations between lots. All detection antibodies are provided separately for maximum flexibility in building custom multiplex assays.

Principle of the Assay

MSD cytokine assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays are all sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots, as shown in the layout below. The assays are provided on MSD 4-spot, 7-spot, or 10-spot SECTOR® plates. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of 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 imager 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.

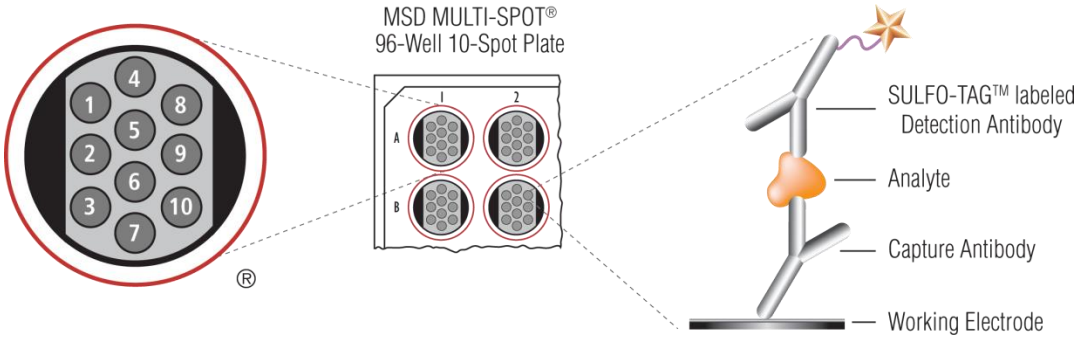

Figure 1: Multiplex plate spot diagram showing possible placement of analyte capture antibodies. A spot map identifying the location of each assay in the special order can be found on the plate packaging. This information will be needed for data analysis. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

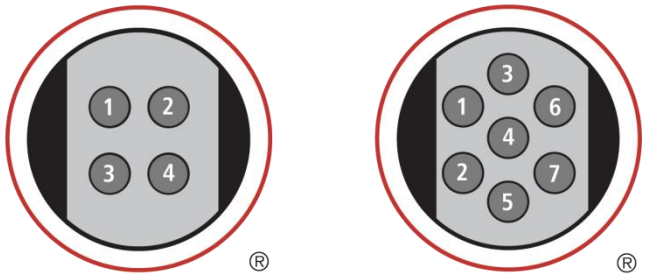

Figure 2: Special order assays can also be provided on 4- or 7-spot plates.

Reagents Supplied

Calibrators:

Calibrators for custom V-PLEX assays are supplied using the same calibrator blends provided with pre-configured V-PLEX kits. Based on the combination of biomarkers chosen from the V-PLEX panels, 1 to 4 multi-analyte calibrators may be supplied. Calibrators for other assays are provided separately.

Detection Antibodies:

Individual SULFO-TAG detection antibodies are provided for each assay ordered.

Diluents:

Diluent 43 (assay diluent) and Diluent 3 (antibody diluent) are the standard diluents supplied with the special order human biomarker group 1. However, to ensure optimum performance, we may provide a different diluent set for custom orders depending on the assays chosen. Diluents will be provided in the 5-plate size. Additional diluent may be purchased for sample dilutions if necessary.

A spot map identifying the location of each assay ordered can be found on the plate packaging. This information will be needed for data analysis.

Reagent	Storage	Catalog #	Description
MULTI-SPOT 96-Well Custom Plate	2–8°C	—	4-, 7-, or 10-spot, 96-well plate, foil sealed, with desiccant.
V-PLEX multi-analyte lyophilized calibrator blend	2–8°C	C0048-2 C0047-2 C0050-2	• Proinflammatory Panel 1 (human) Calibrator Blend • Chemokine Panel 1 (human) Calibrator Blend or Chemokine 9-Analyte (human) Calibrator Blend* • Cytokine Panel 1 (human) Calibrator blend Each blend has multiple recombinant human proteins in diluent, buffered and lyophilized. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).
V-PLEX multi-analyte frozen calibrator blend	≤-70°C	C0190-2	• Angiogenesis Panel 1 (human) Calibrator Blend This blend has multiple recombinant human proteins in diluent. Individual analyte concentration is provided in the lot-specific COA.
Additional individual assay calibrator (50 µg/mL)**	≤-70°C	—	Calibrator for any additional assays, one vial per assay; sufficient for 5 plates.
Diluent 43, Diluent 7, or Diluent 2	≤-10°C	R50AG-2 R51BB-2 R54BB-3	Diluent for samples and calibrator; contains serum, blockers, and preservatives.
Diluent 3	≤-10°C	R51BA-5	Diluent for detection antibody; contains protein, blockers, and preservatives.
Read Buffer T (4X)	RT	R92TC-3	Buffer to catalyze the electrochemiluminescence reaction.
Individual detection antibody for each assay ordered (50X)	2–8°C	—	Individual, anti-human detection antibody, SULFO-TAG conjugated; 1 vial for each assay ordered; sufficient for 5 plates.

*Chemokine 9-Analyte (human) Calibrator Blend is a custom blend without IL-8.

**Calibrator concentration for CA125 is 1250 KU/mL, EPO is 1000 IU/mL, and NT-ProBNP is 15 µg/mL.

Limitations of Use

- IL-12p70 and IL-12/IL-23p40 should not be multiplexed in any combination within the same well because they may interfere with one another.

- IL-8 assays from Proinflammatory Panel 1 and Chemokine Panel 1 [IL-8 and IL-8 (HA)] cannot be multiplexed on the same plate.
- Some assays may require greater dilution (see Suggested Sample Dilutions section in the Appendix).

Required Materials and Equipment (Not Supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog # R61AA-1
- ❑ Adhesive plate seals
- ❑ Deionized water

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 safety data sheet (SDS), which can be obtained from MSD Customer Service.

Best Practices

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific COA.
- Assay incubation steps should be performed between 20-26°C to achieve the most consistent signals between runs.
- Bring frozen diluents to room temperature in a 24°C water bath. Thaw other reagents on wet ice and use as directed.
  The lyophilized calibrators are reconstituted in assay diluent with an incubation for 15 to 30 minutes at room temperature.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid introduction of bubbles, and for empty wells, pipette to the bottom corner.
- Shaking should be vigorous with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.
- Remove the plate seals prior to reading the plate.
- Make sure that Read Buffer T is at room temperature when added to a plate.
- Do not shake the plate after adding Read Buffer T.
- To improve inter-plate precision, keep time intervals consistent between adding Read Buffer T and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding Read Buffer T.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. Partially used plates may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature.

Important: Upon first thaw, aliquot the diluents into suitable volumes before refreezing.

Prepare Calibrator Dilutions

MSD supplies 1 to 4 vials of multi-analyte calibrator blend(s) covering all of the V-PLEX assays and one vial of frozen individual calibrator(s) at 50 µg/mL concentration for each of the other assays on the plate. Please note that bulk calibrators for some analytes like CA125, EPO, and NT-ProBNP are provided at a different concentration. Please verify the concentrations by checking the label on each calibrator vial received. Each vial of calibrator is enough for 5 plates. The concentration of each calibrator is provided in the COA included with the kit.

To prepare calibrator solutions for up to 4 replicates:

1) Reconstitute the lyophilized V-PLEX calibrator(s):
   a) Add 250 µL of Diluent 43 (or other assay diluent) to each of the lyophilized calibrator vials supplied. After reconstituting, invert at least 3 times (do not vortex). Let the reconstituted solution equilibrate at room temperature for 15-30 minutes and then vortex briefly using short pulses.

      Note: Reconstituted calibrator may be stored frozen at ≤-70°C and is stable through 3 freeze–thaw cycles.

      If only lyophilized V-PLEX calibrators are included in the kit, continue to Step 4.
2) Prepare the frozen V-PLEX calibrator:
   a) Thaw the frozen V-PLEX calibrator on wet ice for at least 30 minutes and keep on ice.

      If no additional individual frozen calibrators are included in the kit, continue to Step 4.
3) Prepare a working stock of each individual frozen calibrator.
   a) Thaw all frozen calibrators on wet ice for at least 30 minutes and keep on ice.
   b) Locate your analytes in either Table 1a or Table 1b on the following page and note the table in which they are listed. Analytes from Table 1a require an additional dilution step. See **Example: Calibrator Dilution** section in the Appendix.
   c) **Biomarkers listed in Table 1a:** Prepare a 1 µg/mL intermediate stock of each calibrator by mixing 10 µL of each bulk calibrator with 490 µL of Diluent 43 (or assay diluent supplied). Then dilute the intermediate stock as shown in Table 1a to create a 50X working stock. Separate the working stock into aliquots and store any calibrator not immediately needed at ≤-70°C. Stock calibrator may be refrozen and thawed once.

Human Cytokine Assays	Intermediate Stock (µL)	Assay diluent (µL)	50X Working Stock Concentration (µg/mL)
β-NGF	50	350	0.125
EGF	10	390	0.025
FAS Ligand	50	350	0.125
G-CSF	50	350	0.125
GRO-α	50	350	0.125
I-309	100	300	0.25
ICAM-3	50	350	0.125
IFN-α2a	50	350	0.125
IL-6R	50	350	0.125
IL-17B	100	300	0.25
IL-17D	100	300	0.25
IL-18	50	350	0.125
I-TAC	50	350	0.125
MIG	50	350	0.125

Table 1a: Preparation of 50X Working Stocks

d. **Biomarkers listed in Table 1b.** Dilute each thawed bulk calibrator to the 50X working stock concentration as shown below. Separate the working stock into aliquots and store any calibrator not immediately needed at ≤-70°C.

Other Human Cytokine Assays	Bulk Calibrator (µL)	Assay Diluent (µL)	50X Working Stock Concentration (µg/mL)
BDNF	20	180	5
CA-125	25	100	250 KU/mL
Calbindin	10	390	1.25
CTACK	12	788	0.75
EPO	80	80	500 IU/mL
E-Selectin	10	990	0.5
Fractalkine	20	180	5
MCP-2	10	990	0.5
MCP-3	10	990	0.5
M-CSF	10	990	0.5
MIP-3α	10	990	0.5
MIP-3β	10	990	0.5
MIP-5	10	990	0.5
MMP-1	20	180	5.0
MMP-2	80	80	25
MMP-3	20	180	5.0
MMP-9	80	80	25
MMP-10	20	180	5.0
NT-ProBNP	10	290	0.5
Osteoactivin	10	240	2.0
Osteonectin	20	180	5.0
OPGN	20	180	5.0
Osteopontin	20	180	5.0
P-Selectin			Provided at 50X (50 µg/mL)
RANTES	10	990	0.5
SDF-1α	10	990	0.5
TNF-RI	10	990	0.5
TNF-RII	10	990	0.5
TPO	10	990	0.5
TRAIL	10	490	1.0
YKL-40	10	190	2.5

Table 1b: Preparation of 50X Working Stocks

4) Prepare 7 calibrator solutions plus a zero calibrator blank:

   a) Prepare Calibrator 1 by combining 200 µL of each lyophilized V-PLEX calibrator blend from Step 1, 40 µL of frozen V-PLEX calibrator blend from Step 2, and 16 µL of each calibrator(s) from Step 3. Then add Diluent 43 (or assay diluent) to bring this to a final volume of 800 µL. Mix well by vortexing.

   b) Prepare Calibrator 2 by transferring 100 µL of Calibrator 1 to 300 µL of Diluent 43 (or assay diluent). Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.

   c) Use Diluent 43 (or assay diluent) as Calibrator 8 (zero calibrator).

   Note: You may aliquot Calibrator 1 and store at ≤-70°C for 1 month or at 2–8°C for 5 days. Non-V-PLEX calibrators may not have been validated for stability. Discard any unused, diluted calibrators.

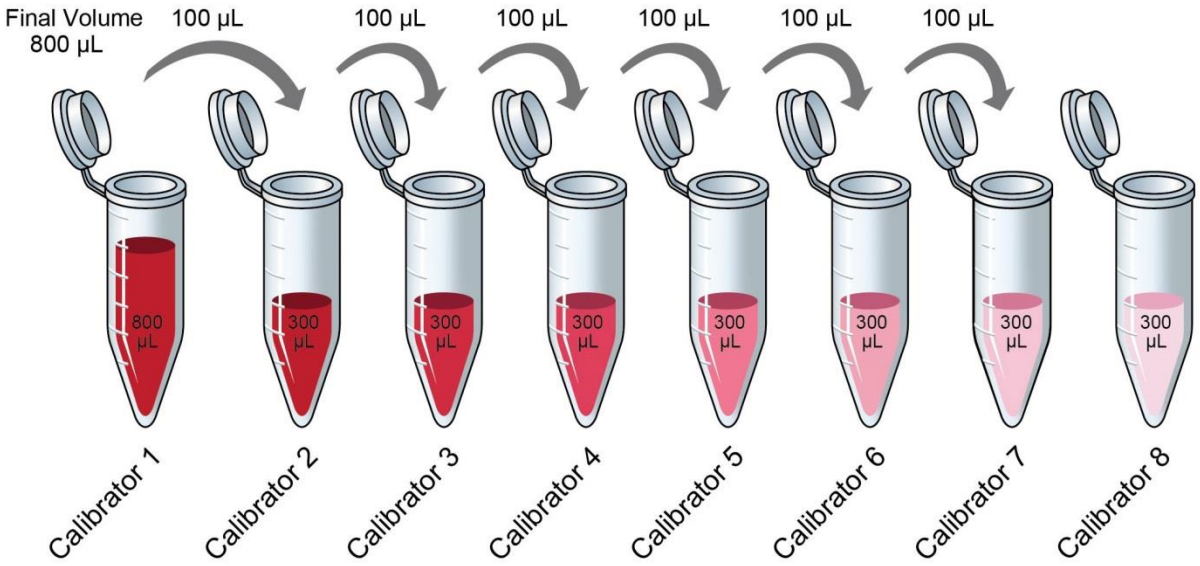

Figure 3: Typical dilution cascade. Adjust Calibrator 1 for assays ordered.

Suggested Top Concentrations for Calibration Curve

V-PLEX assays: For the lot-specific concentration of each calibrator in the multi-analyte calibrator blend, refer to the COA supplied with the kit.

Other selected assays: The calibration curve used in the assay will be dependent on the assays and reagents. The calibration curve can be adjusted as needed. Suggested top-of-the-curve concentrations and dilution factors for MSD human cytokine assays are provided below.

Other Assays	Calibrator 1 concentration (pg/mL)	Serial Dilution Factor
BDNF	100,000	4
β-NGF	2,500	4
CA-125	5,000 U/mL	4
Calbindin	25,000	4
CTACK	15,000	4
EGF	500	4
EPO	10,000 mIU/mL	4
E-Selectin	10,000	4
FAS Ligand	2,500	4
Fractalkine	100,000	4
G-CSF	2,500	4
GRO-α	2,500	4
I-309	5,000	4
ICAM-3	2,500	4
IFN-α2a	2,500	4
IL-6R	2,500	4
IL-17B	5,000	4
IL-17D	5,000	4
IL-18	2,500	4
I-TAC	2,500	4
MCP-2	10,000	4
MCP-3	10,000	4
M-CSF	10,000	4
MIG	2,500	4
MIP-3α	10,000	4
MIP-3β	10,000	4
MIP-5	10,000	4
MMP-1	100,000	4
MMP-2	500,000	4
MMP-3	100,000	4
MMP-9	500,000	4
MMP-10	100,000	4
NT-ProBNP	10,000	4
Osteoactivin	40,000	4
Osteonectin	100,000	4
OPGN	100,000	4
Osteopontin	100,000	4
P-Selectin	1,000,000	4
RANTES	10,000	4
SDF-1α	10,000	4
TNF-RI	10,000	4
TNF-RII	10,000	4
TPO	10,000	4
TRAIL	20,000	4
YKL-40	50,000	4

Table 2: Calibrator 1 concentrations for selected assays

Dilute Samples

Dilute samples with Diluent 43 (or assay diluent). For human serum, plasma, and urine samples, MSD recommends a minimum 2-fold dilution. For example, to dilute 2-fold, add 60 µL of sample to 60 µL of Diluent 43 (or assay diluent). You may conserve sample volume by using a higher dilution.

Some assays may require greater dilution (see Suggested Sample Dilutions section in the Appendix).

The appropriate dilution for other sample matrices may need to be determined. The dilution for supernates may require additional dilution based on stimulation and analyte concentrations in the sample. A 2-fold dilution may be an appropriate starting point.

Prepare Detection Antibody Solution

MSD provides each detection antibody separately as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use.

For one plate, combine 60 µL of each supplied 50X detection antibodies, then add Diluent 3 to bring the final volume to 3,000 µL.

Prepare Wash Buffer

MSD recommends using MSD Wash Buffer (catalog # R61AA-1) or PBS + 0.05% Tween-20 for plate washing. MSD Wash Buffer is provided as a 20X stock solution. The working solution is 1X.

For one plate, combine:

- ❑ 15 mL of Wash Buffer (20X)
- ❑ 285 mL of deionized water

Prepare Read Buffer T

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For one plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may keep excess diluted Read Buffer T in a tightly sealed container at room temperature for up to one month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

Assay Protocol

1. **Wash* and Add Sample:** Wash the plate 3 times with at least 150 µL/well of wash buffer.

   ***Note:** Pre-washing the plate with wash buffer prior to sample addition may provide greater uniformity of results for certain assays. MSD's V-PLEX kit QC procedure includes this pre-wash; however, it can be considered as an optional step. After performing this pre-wash step, the recommended protocol outlined below can be followed.

2. **Add Sample:** Add 50 µL of diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

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 and incubate at room temperature with shaking for 2 hours.

4. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of wash buffer. Add 150 µL of 2X Read Buffer T to each well. Read the plate on the MSD instrument. Incubation in read buffer is not required before reading the plate.

Alternate Protocols

The suggestions below may be useful as alternate protocols; however, they have not been tested by MSD Quality Control.

- **Alternate Protocol 1, Extended Sample Incubation:** Incubating samples overnight at 2–8ºC may improve sensitivity for some assays. Shaking may improve performance.
- **Alternate Protocol 2, Single Wash (Tissue Culture):** For tissue culture samples, you may simplify the protocol by eliminating one of the wash steps. After incubating diluted sample, calibrator, or control, add detection antibody solution to the plate without decanting or washing the plate.
- **Alternate Protocol 3, Dilute-in-Plate:** To limit sample handling, you may dilute samples and controls in the plate. For 2-fold dilution, add 25 µL of assay diluent to each sample/control well, and then add 25 µL of neat control or sample. Calibrators should not be diluted in the plate; add 50 µL of each calibrator directly into empty wells.

Assay Components

Antibodies: V-PLEX assays

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
bFGF	Mouse Monoclonal	Mouse Monoclonal	A
Eotaxin	Mouse Monoclonal	Mouse Monoclonal	B
Eotaxin-3	Mouse Monoclonal	Mouse Monoclonal	B
Flt-1	Mouse Monoclonal	Goat Polyclonal	A
GM-CSF	Mouse Monoclonal	Rat Monoclonal	A
IFN-γ	Mouse Monoclonal	Mouse Monoclonal	C
IL-1α	Mouse Monoclonal	Goat Polyclonal	A
IL-1β	Mouse Monoclonal	Goat Polyclonal	B
IL-2	Mouse Monoclonal	Mouse Monoclonal	B
IL-4	Mouse Monoclonal	Mouse Monoclonal	B
IL-5	Mouse Monoclonal	Rat Monoclonal	B
IL-6	Mouse Monoclonal	Goat Polyclonal	C
IL-7	Mouse Monoclonal	Goat Polyclonal	A
IL-8	Mouse Monoclonal	Goat Polyclonal	B
IL-8 (HA)	Mouse Monoclonal	Goat Monoclonal	C
IL-10	Mouse Monoclonal	Mouse Monoclonal	B
IL-12/IL-23p40	Mouse Monoclonal	Mouse Monoclonal	B
IL-12p70	Mouse Monoclonal	Mouse Monoclonal	B
IL-13	Rat Monoclonal	Mouse Monoclonal	B
IL-15	Mouse Monoclonal	Mouse Polyclonal	A
IL-16	Mouse Monoclonal	Goat Polyclonal	A
IL-17A	Mouse Monoclonal	Goat Polyclonal	A
IP-10	Mouse Monoclonal	Mouse Monoclonal	B
PlGF	Mouse Monoclonal	Mouse Monoclonal	B
MCP-1	Mouse Monoclonal	Mouse Monoclonal	B
MCP-4	Mouse Monoclonal	Mouse Monoclonal	B
MDC	Mouse Monoclonal	Mouse Monoclonal	B
MIP-1α	Mouse Monoclonal	Mouse Monoclonal	B
MIP-1β	Mouse Monoclonal	Mouse Monoclonal	B
TARC	Mouse Monoclonal	Mouse Monoclonal	B
Tie-2	Mouse Monoclonal	Goat Polyclonal	A
TNF-α	Mouse Monoclonal	Goat Polyclonal	B
TNF-β	Mouse Monoclonal	Mouse Monoclonal	A
VEGF-A	Mouse Monoclonal	Mouse Monoclonal	C
VEGF-C	Mouse Monoclonal	Goat Polyclonal	A
VEGF-D	Mouse Monoclonal	Goat Polyclonal	A

Table 3: Antibodies for V-PLEX assays

Antibodies: Other Assays

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
BDNF	Mouse Monoclonal	Mouse Monoclonal	A
β-NGF	Mouse Monoclonal	Goat Polyclonal	A
CA-125	Mouse Monoclonal	Mouse Monoclonal	A
Calbindin	Mouse Monoclonal	Goat Polyclonal	A
CTACK	Mouse Monoclonal	Goat Polyclonal	A
EGF	Mouse Monoclonal	Goat Polyclonal	A
EPO	Mouse Monoclonal	Mouse Monoclonal	A

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
E-Selectin	Mouse Monoclonal	Mouse Monoclonal	A
FAS Ligand	Mouse Monoclonal	Mouse Monoclonal	A
Fractalkine	Mouse Monoclonal	Goat Polyclonal	A
G-CSF	Mouse Monoclonal	Goat Polyclonal	A
GRO-α	Mouse Monoclonal	Goat Polyclonal	A
I-309	Mouse Monoclonal	Goat polyclonal	A
ICAM-3	Mouse Monoclonal	Mouse Monoclonal	A
IFN-α2a	Mouse Monoclonal	Mouse Monoclonal	A
IL-6R	Mouse Monoclonal	Goat Polyclonal	A
IL-17B	Mouse Monoclonal	Mouse Monoclonal	A
IL-17D	Mouse Monoclonal	Goat Polyclonal	A
IL-18	Mouse Monoclonal	Rat Monoclonal	A
I-TAC	Mouse Monoclonal	Mouse Monoclonal	A
MCP-2	Mouse Monoclonal	Mouse Monoclonal	A
MCP-3	Mouse Monoclonal	Goat Monoclonal	A
M-CSF	Mouse Monoclonal	Goat Polyclonal	A
MIG	Mouse Monoclonal	Goat Polyclonal	A
MIP-3α	Mouse Monoclonal	Rabbit Polyclonal	A
MIP-3β	Goat Polyclonal	Goat Polyclonal	A
MIP-5	Mouse Monoclonal	Goat Polyclonal	A
MMP-1	Goat Polyclonal	Goat Polyclonal	A
MMP-2	Goat Polyclonal	Goat Polyclonal	A
MMP-3	Goat Polyclonal	Goat Polyclonal	A
MMP-9	Mouse Monoclonal	Goat Polyclonal	A
MMP-10	Mouse Monoclonal	Goat Polyclonal	A
NT-ProBNP	Mouse Monoclonal	Mouse Monoclonal	A
Osteoactivin	Goat Polyclonal	Goat Polyclonal	A
Osteonectin	Goat Polyclonal	Mouse Monoclonal	A
OPGN	Goat Polyclonal	Mouse Monoclonal	A
Osteopontin	Goat Polyclonal	Goat Polyclonal	B
P-Selectin	Mouse Monoclonal	Mouse Monoclonal	A
RANTES	Mouse Monoclonal	Goat Polyclonal	A
SDF-1α	Mouse Monoclonal	Goat Polyclonal	A
TNF-RI	Mouse Monoclonal	Goat Polyclonal	A
TNF-RII	Mouse Monoclonal	Goat Polyclonal	A
TPO	Goat Polyclonal	Goat Polyclonal	A
TRAIL	Mouse Monoclonal	Goat Polyclonal	A
YKL-40	Mouse Monoclonal	Rabbit Polyclonal	A

Table 4: Antibodies for selected assays

Certificate of Analysis for Special Order Cytokine Assays (Human)

The COA for Human Cytokine Special Order Assays will include the following:

- Signal and %CV from calibration curve for each calibrator
- Background signal
- Non-specific binding for all analytes (individual detection antibodies tested with blended calibrator)
- List of components with lot numbers used for QC testing

Appendix

Example: Calibrator Dilution

In this example, the Special Order kit contains IL-2, IL-4, IL-6, GM-CSF, IL-17A, GRO-α, and Fractalkine. The kit is provided with the Proinflammatory Panel 1 (human) lyophilized calibrator blend, Cytokine Panel 1 (human) lyophilized calibrator blend, and individual calibrators for human GRO-α and Fractalkine.

STEP 1: Reconstitute/Thaw Calibrators

- ❑ Reconstitute each of the lyophilized calibrator with 250 µL of Diluent 43. Mix by inverting at least 3 times. Equilibrate at room temperature for 15-30 minutes and vortex briefly using short pulses.
- ❑ Thaw GRO-α and Fractalkine calibrators on wet ice.

STEP 2: Prepare the Working Stocks

- ❑ For GRO-α, prepare a 1 µg/mL intermediate stock by mixing 10 µL of the 50 µg/mL bulk calibrator with 490 µL of Diluent 43. Then prepare a 50X working stock by adding 50 µL of the intermediate stock to 350 µL of Diluent 43 (see Table 1a).
- ❑ For Fractalkine, prepare the 50X working stock by adding 20 µL of 50 µg/mL bulk calibrator to 180 µL of Diluent 43 (see Table 1b). Aliquot the working stocks into 5 vials and store unused portions at ≤-70°C.

STEP 3: Prepare the Blended Calibrator

- ❑ Combine 200 µL of each reconstituted V-PLEX calibrator into one vial. Add 16 µL each of GRO-α and Fractalkine working stock to the vial containing the combined reconstituted V-PLEX calibrators. Add 368 µL of Diluent 43 to bring the blended calibrator to a volume of 800 µL. Mix well by vortexing.
- ❑ After following the instructions above, the blended calibrator concentration should be at the suggested top of curve. The concentration of GRO-α in the vial should be 2,500 pg/mL (see Table 2). The concentration of Fractalkine in the vial should be 100,000 pg/mL. The concentration of IL-2, IL-4, IL-6, GM-CSF, and IL-17A are provided in the COA.

STEP 4: Prepare Calibrator Dilutions

- ❑ To prepare calibrator for up to 4 replicates, use the blended calibrator prepared in Step 3 as Calibrator 1, then transfer 100 µL of Calibrator 1 into 300 µL of Diluent 43.
- ❑ Repeat the 4-fold serial dilution 6 times to generate calibrators 2 through 7. Use Diluent 43 as Calibrator 8.

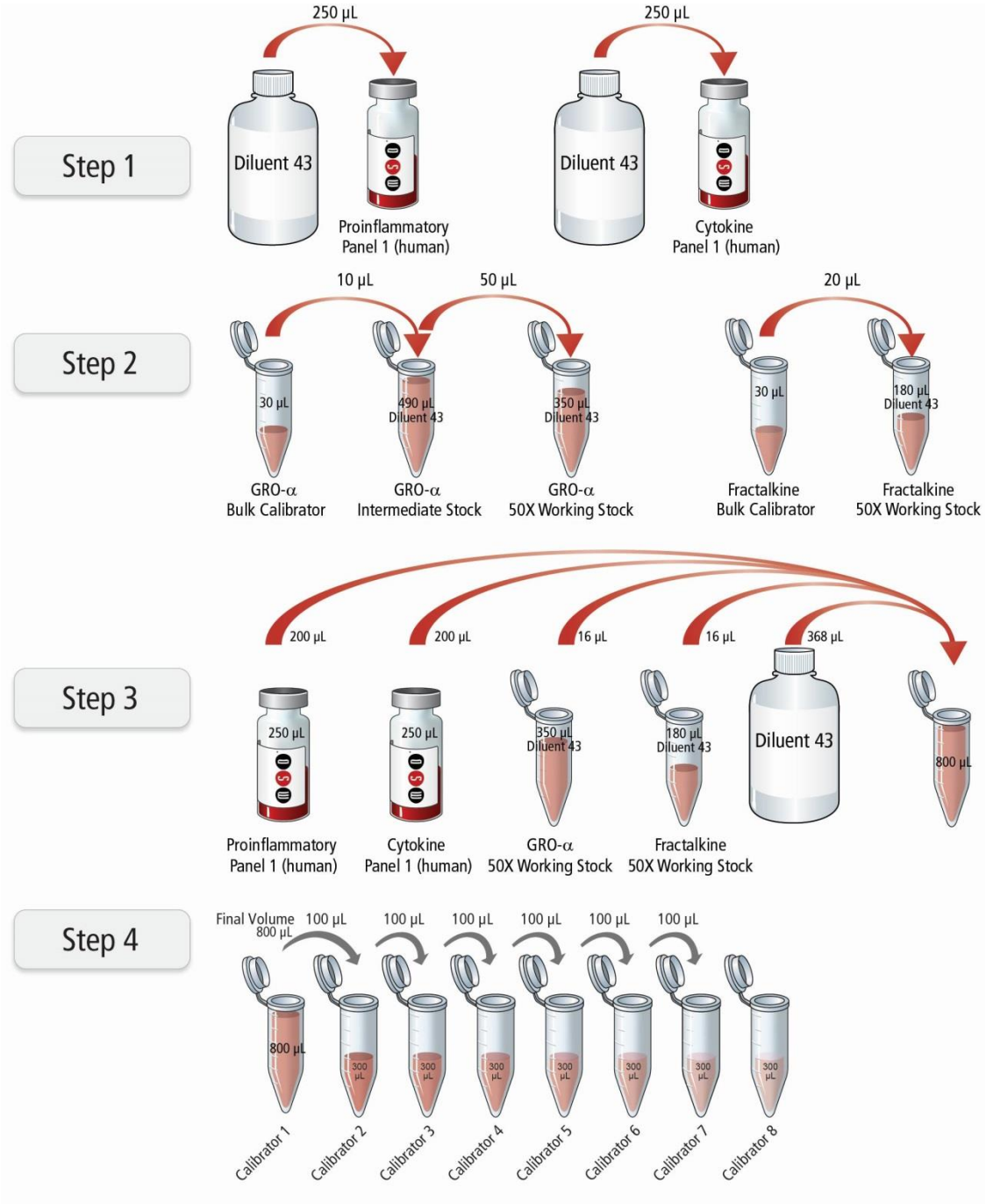

Figure 4. Calibrator Dilution Example for the Special Order Kit containing IL-2, IL-4, IL-6, GM-CSF, IL-17A, GRO-α, and Fractalkine

Suggested Sample Dilutions

The suggested 2-fold dilution may not be appropriate for all samples and study conditions. These suggestions were determined from normal controls across multiple studies. In some cases, a greater dilution was suggested because the assay is present on a panel where other assays require a greater dilution. An example of this is the Chemokine Panel 1 (human) where several assays can be run at 2-fold dilution, but the panel is run at 4-fold dilution. You should pick the appropriate dilution for your study. If a dilution is not listed below, a suggestion dilution has not been developed. The suggested dilution factors are based on abundance of the analyte in the sample, not on matrix effects.

		Suggested Fold Dilution		
	Assay	Serum/Plasma	Urine	CSF
Proinflammatory Panel 1 (human)	IFN-γ	2	2	2
	IL-1β	2	2	2
	IL-2	2	2	2
	IL-4	2	2	2
	IL-6	2	2	2
	IL-8	2	2	2
	IL-10	2	2	2
	IL-12p70	2	2	2
	IL-13	2	2	2
	TNF-α	2	2	2
Chemokine Panel 1 (human)	Eotaxin	4	4	4
	MIP-1β	4	4	4
	Eotaxin-3	4	4	4
	TARC	4	4	4
	IP-10	4	4	4
	MIP-1α	4	4	4
	IL-8 (HA)	4	4	4
	MCP-1	4	4	4
	MDC	4	4	4
	MCP-4	4	4	4
Cytokine Panel 1 (human)	GM-CSF	2	2	2
	IL-1α	2	2	2
	IL-5	2	2	2
	IL-7	2	2	2
	IL-12/IL-23p40	2	2	2
	IL-15	2	2	2
	IL-16	2	2	2
	IL-17A	2	2	2
	TNF-β	2	2	2
	VEGF-A	2	2	2
Angiogenesis Panel 1(human)	VEGF-C	2	2	2
	VEGF-D	2	2	2
	Tie-2	2	2	2
	Flt-1	2	2	2
	PlGF	2	2	2
	bFGF	2	2	2
	BDNF	2	2	2
	β-NGF	2	2	2
	CA-125	2	2	2
	Calbindin	10	10	

	Suggested Fold Dilution		
Assay	Serum/Plasma	Urine	CSF
CTACK	2	2	2
EGF	2	2	2
EPO	2	2	2
E-Selectin	2	2	
FAS Ligand	2	2	2
Fractalkine	2		
G-CSF	2	2	2
GRO-α	2	2	2
I-309	2	2	2
ICAM-3	2		
IFN-α2a	2	2	2
IL-6R	50		
IL-17B	2	2	2
IL-17D	2	2	2
IL-18	2	2	2
I-TAC	2	2	2
MCP-2	2	2	2
MCP-3	2	2	2
M-CSF	2	2	2
MIG	2	2	2
MIP-3α	2	2	2
MIP-3β	2	2	2
MIP-5	10		
MMP-1	10		
MMP-2	2		
MMP-3	10		
MMP-9	10		
MMP-10	2		
NT-ProBNP	2	2	
OPGN	2	2	
Osteoactivin	10	10	
Osteonectin	2	2	
Osteopontin	2	2	
P-Selectin	2	2	2
RANTES	50		
SDF-1α	2	2	2
TNF-RI	10		
TNF-RII	10		
TPO	50		
TRAIL	2	2	

Table 5: Suggested sample dilutions