MSD® MULTI-SPOT Assay System

Special Order NHP Biomarker Group 1 Kit

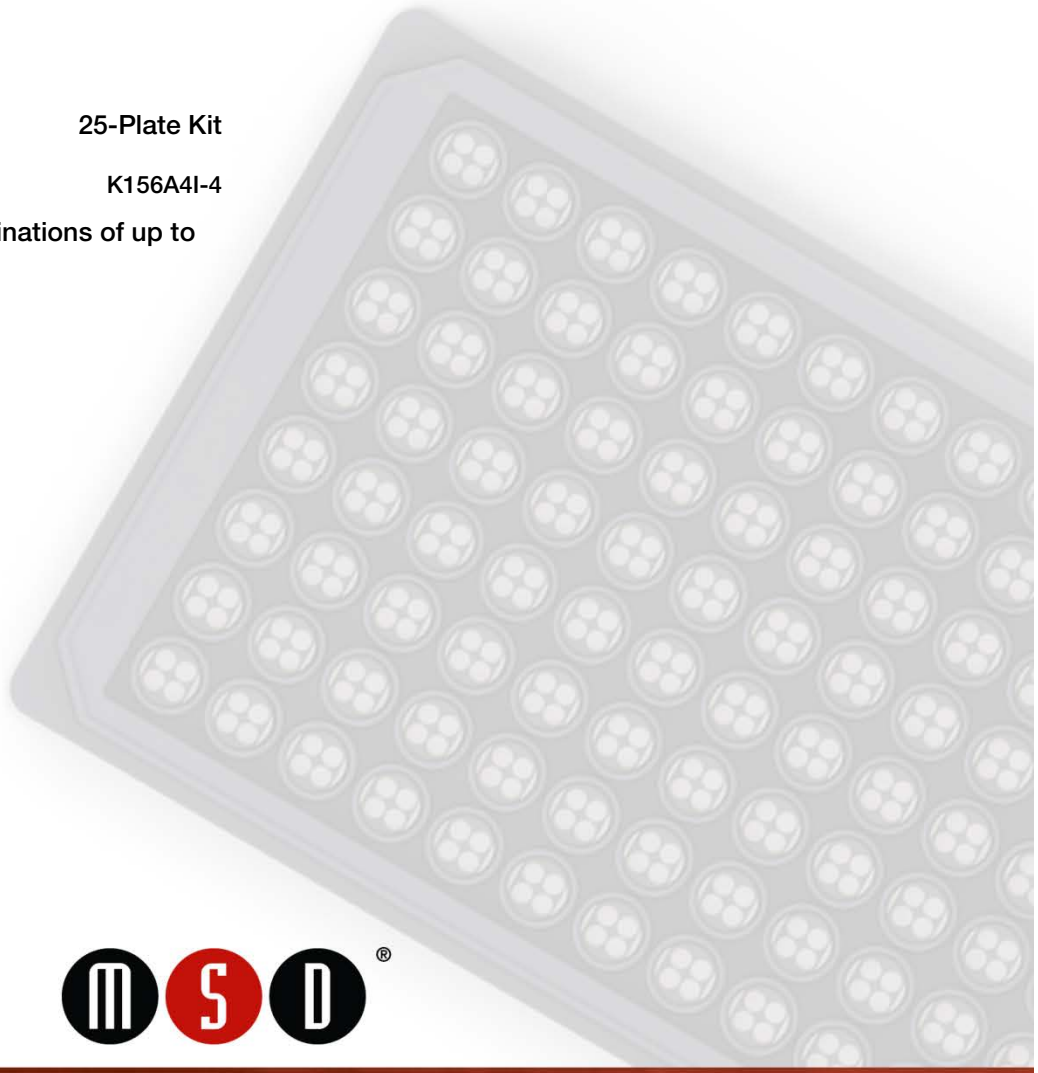
V-PLEX® assays: Eotaxin-3, bFGF, Flt-1, GM-CSF, IFN-γ, IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8, IL-8 (HA*), IL-10, IL-12/IL-23p40,IL-15, IL-16, IL-17A, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PIGF, TARC, Tie-2, TNF-β, VEGF-A, VEGF-D

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

Other MSD catalog NHP assays: CA-125, CRP, Eotaxin, EPO, E-Selectin, Fractalkine, G-CSF, IL-13, IL-17D, I-TAC, MCP-2, M-CSF, Osteocalcin, Osteopontin, Osteoprotegerin, RANTES, Thrombomodulin, TIMP-1, TNF-RII, TPO, TRAIL

Special Order Kits	5-Plate Kit	25-Plate Kit
Catalog #	K156A4I-2	K156A4I-4

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



MSD Cytokine Assays

Special Order NHP Biomarker Group 1 Kit

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

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

Other MSD Catalog NHP assays: CA-125, CRP, Eotaxin, EPO, E-Selectin, Fractalkine, G-CSF, IL-13, IL-17D, I-TAC, MCP-2, M-CSF, Osteocalcin, Osteopontin, Osteoprotegerin, RANTES, Thrombomodulin, TIMP-1, TNF-RII, TPO, TRAIL

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. The assays listed below have been tested by MSD for analytical compatibility. Data on analytical performance, calibration curves, reproducibility, and specificity are provided in the certificate of analysis (C of A) 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 NHP Biomarker Group 1 Kit are:

MSD NHP V-PLEX Assays

Proinflammatory Panel 1: IFN-γ, IL1-β, IL-2, IL-6, IL-8, IL-10
Chemokine Panel 1: MIP-1β, Eotaxin-3, TARC, IP-10, MIP-1α, IL-8 (HA*), MCP-1, MDC, MCP-4
Cytokine Panel 1: GM-CSF, IL-5, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, VEGF-A
Angiogenesis Panel 1: bFGF, Flt-1, PlGF, Tie-2, VEGF-D

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

Other Analytically Compatible MSD NHP Assays

CA 125, CRP, Eotaxin, EPO, E-Selectin, Fractalkine, G-CSF, IL-13, IL-17D, I-TAC, MCP-2, M-CSF, Osteocalcin, Osteopontin, Osteoprotegerin, RANTES, Thrombomodulin, TIMP-1, TNF-RII, TPO, TRAIL

The Special Order NHP Biomarker Group 1 Kit contains plates pre-coated with specific capture antibodies. Other reagents (calibrators and detection antibodies) are provided based on the assays selected. Reagents for special order V-PLEX assays are the same as those provided in the pre-configured V-PLEX kits. Calibrators for the standard assays are supplied at high concentrations to provide ample material for blending with the multi-analyte V-PLEX calibrator(s). All detection antibodies are provided separately for maximum flexibility in building custom multiplex assays.

The assays provided in special order kits measure biomarkers produced by non-human primates (NHP). These assays have been tested by measuring either endogenous analytes in NHP samples (e.g., serum, plasma, urine, or CSF) or analytes produced by cultured NHP cells (e.g., cytokines produced by stimulated PBMCs *in vitro*).

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.

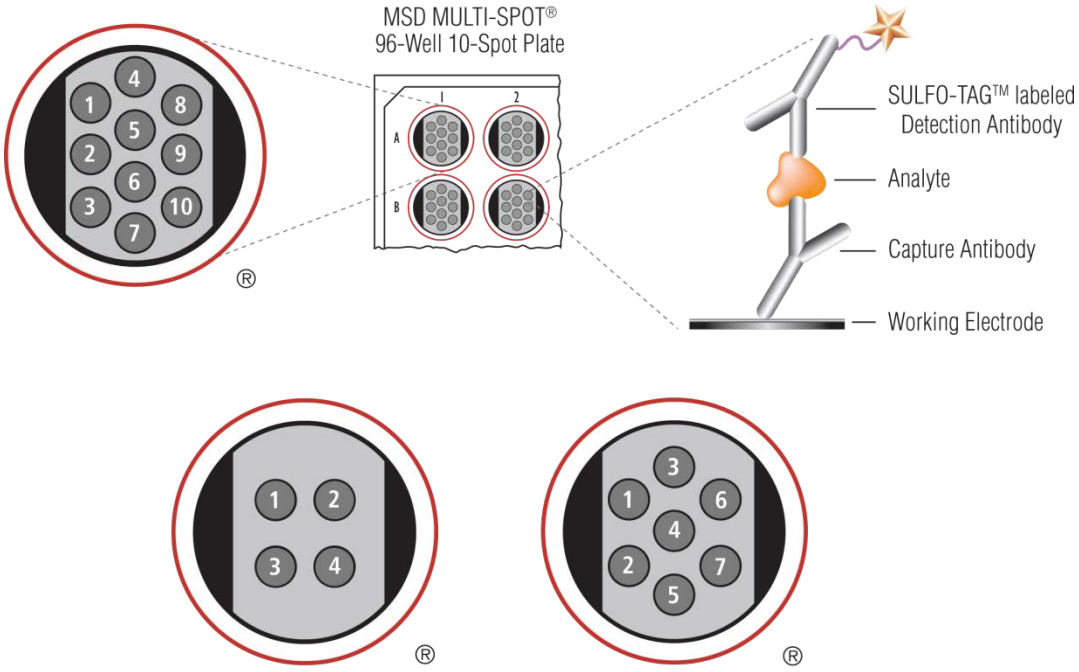


Figure 1: Multiplex plate spot diagram showing possible placement of analyte capture antibodies. A spot map identifying the location of each assay in the special order can be found on the plate packaging. This information will be needed for data analysis. The numbering convention for the different spots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

Reagents Supplied

Calibrators:

The assays use human recombinant biomarker calibrator sequences that are highly homologous to the non-human primate biomarkers. Calibrators for the validated V-PLEX assays are supplied as multi-analyte calibrator blends. Based on the combination of biomarkers chosen from the V-PLEX panels, 1 to 4 multi-analyte calibrators may be supplied. Individual calibrators are provided for standard assays.

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 NHP Biomarker Group 1 Kit. 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 from MSD for sample dilutions if necessary.

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	Depending on the V-PLEX assays ordered,1, 2 or all of the following may be provided: • Proinflammatory Panel 1 (human) Calibrator Blend • Chemokine Panel 1 (human)Calibrator Blend • Chemokine 9-Analyte (human) Calibrator Blend* • Cytokine Panel 1 (human) Calibrator Blend Each blend has multiple recombinant proteins in diluent, buffered and lyophilized. Individual analyte concentration is provided in the lot-specific C of A.
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 C of A.
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 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 and EPO is 1000 IU/mL

Limitations of Use

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

- Note that the VEGF-A assay is part of both the cytokine and angiogenesis panels, both of which use blended calibrators. Therefore, if your multiplex includes VEGF-A along with assays from both the cytokine and angiogenesis panels, the concentration curve should be calculated based on the combined concentration of VEGF-A in both blended calibrators.

Additional Materials and Equipment

- ❑ 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 300–1000 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 and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific certificate of analysis (COA).
- 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.
- 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 300 and 1000 rpm.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove plate seals prior to reading the plate.
- If assay results are above the top of the calibration curve, dilute samples, and repeat the assay.
- When running a partial plate, seal the unused sectors (see sector map in instrument and software manuals) 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, separate the diluents into aliquots appropriate for the size of your needs before refreezing. After thawing serum-based diluent such as Diluent 43, a precipitate may appear in the solution. Mix or vortex the diluent and proceed with the assay. Any remaining precipitate will not compromise assay performance.

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 and EPO are provided at a different concentration. Each vial of calibrator is enough for 5 plates. The concentration of each calibrator is provided in the C of A included with the kit.

To prepare calibrator solutions for up to 4 replicates:

1) Reconstitute the lyophilized V-PLEX calibrator blend(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 is not stable when stored at 2–8°C; however, it 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 ice, then vortex.

   Note: Undiluted frozen calibrator may be stored at ≤-70°C and may be refrozen and thawed once.

   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 calibrators on ice then vortex.

   b) Locate your analytes in either Table 1a or Table 1b below and note the table in which they are listed. Analytes from Table 1a require an intermediate dilution step before dilution. 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 below to create a 50X working stock. Separate the working stock into aliquots and store any calibrator not immediately needed at ≤-70°C.

Table 1a: Preparation of 50X Working Stocks

Assays	Intermediate Stock (µL)	Assay diluent (µL)	50X Working Stock Concentration (µg/mL)
IL-17D	100	300	0.25
I-TAC	50	350	0.125

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

Table 1b: Preparation of 50X Working Stocks

Assays	Bulk Calibrator (µL)	Assay Diluent (µL)	50X Working Stock Concentration (µg/mL)
CA-125	25	100	250 KU/mL
CRP	30	0	50
Eotaxin	10	990	0.5
EPO	80	80	500 IU/mL
E-Selectin	20	180	5
Fractalkine	20	180	5
G-CSF	10	990	0.5
IL-13	10	990	0.5
MCP-2	10	990	0.5
M-CSF	10	990	0.5
Osteocalcin	20	80	10
Osteopontin	20	180	5.0
Osteoprotegerin	20	80	10
RANTES	10	990	0.5
Thrombomodulin	30	0	50
TIMP-1	10	240	2
TNF-RII	10	990	0.5
TPO	10	990	0.5
TRAIL	10	490	1.0

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 the blend to a final volume of 800 µL.

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

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

d) 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.

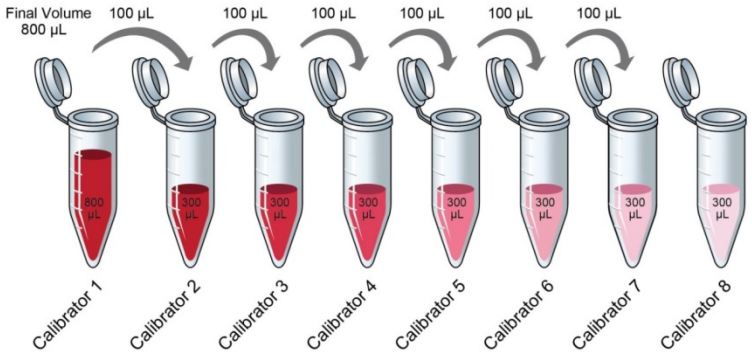


Figure 2: Typical dilution cascade. Adjust Calibrator 1 for assays ordered.

Suggested Top Concentrations for Calibration Curve

V-PLEX assays: For the lot-specific concentration of each calibrator in the multi-analyte calibrator blends, refer to the C of A supplied with the kit. If your multiplex includes VEGF-A along with other assays from both the cytokine/chemokine and angiogenesis panels, the Calibrator 1 concentration for VEGF-A is the combined concentrations of VEGF-A in both blended calibrators.

Selected standard 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.

	Calibrator 1 concentrations for selected assays	
Other Assays	Calibrator 1 concentration (pg/mL)	Serial Dilution Factor
CA-125	5,000 U/mL	4
CRP	1,000 000	4
Eotaxin	10,000	4
EPO	10,000 mIU/mL	4
E-Selectin	100,000	4
Fractalkine	100,000	4
G-CSF	10,000	4
IL-13	10,000	4
IL-17D	5,000	4
I-TAC	2,500	4
MCP-2	10,000	4
M-CSF	10,000	4
Osteocalcin	200,000	4
Osteopontin	100,000	4
Osteoprotegerin	200,000	4
RANTES	10,000	4
Thrombomodulin	1,000,000	4
TIMP-1	40,000	4
TNF-RII	10,000	4
TPO	10,000	4
TRAIL	20,000	4

Table 2: Calibrator 1 concentrations for selected assays

Dilute Samples

Dilute samples with Diluent 43 (or assay diluent). For NHP serum, plasma, and urine samples, MSD recommends a minimum 2-fold dilution; however, you may adjust dilution factors for the sample set under investigation. 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). BSA, tissue culture media, or MSD diluent may be used for the initial dilution. Additional MSD diluent is available at www.mesoscale.com.

The appropriate dilution for other sample matrices may need to be determined. Supernatant 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 1 plate, combine 60 µL of each supplied 50X detection antibody, then add Diluent 3 to bring the final volume to 3000 µL.

Prepare Wash Buffer

MSD recommends using phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog # R61AA-1. MSD Wash Buffer is provided as a 20X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 15 mL of wash buffer (20X)
- ❑ 285 mL of deionized water

Prepare Read Buffer

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

For 1 plate, combine:

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

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 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.

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.

Alternate Protocols

The suggestions below may be useful for simplifying the protocol; however, they have not been tested for assays that are not part of the V-PLEX product line.

- **Alternate Protocol 1, Extended Sample Incubation:** Incubating samples overnight at 2–8°C 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.

Cross-Reactivity

Peripheral blood mononuclear cells (PBMC) from rhesus or cynomolgus monkeys were incubated at 37°C with or without a stimulant (lipopolysaccharide [LPS], phytohaemagglutinin [PHA], pokeweed mitogen [PWM], or concanavalin A [Con A]). Changes in cytokine production were assessed at 6, 24, and 48 hours. Specifically, we assessed changes either occurring spontaneously during cell culture or induced by stimulants. The table below summarizes the maximum changes in cytokine production observed at 6, 24, or 48 hours [+++, ++, and + indicate >100, >10, and >2 fold response, respectively; – indicates no significant response]. When analytes were initially undetectable, calculations for fold increase were based on the assay's LLOD. At each time point, fold changes were calculated by normalizing the stimulated levels to cytokine levels from untreated controls rather than background levels from unconditioned cell culture media.

	Cynomolgus Monkey					Rhesus Monkey				
	Con A	LPS	PHA	PWM	Spontaneous	Con A	LPS	PHA	PWM	Spontaneous
GM–CSF	+++	+	+++	+++	–	+	+	+	++	–
IL–5	++	–	+++	++	–	–	–	+	–	–
IL–7	–	–	+	+	–	–	–	–	–	–
IL–12p40	+	–	–	+	–	–	+	–	+	–
IL–15	–	–	–	–	+	–	–	–	+	++
IL–16	–	+	+	+	–	–	+	+	–	++
IL–17	++	+	++	++	–	+++	+	+++	+++	+
TNF–β	+++	+	+++	+++	–	+++	+	++	+++	–
VEGF-A	+	+	+	+	++	–	+	+	+	–
IFN–γ	+++	–	+++	+++	–	+++	+	+++	++	+
IL–1β	–	+	+	+	–	+	+	+	+	–
IL–2	++	+	++	++	+	++	+	++	+	+
IL–6	+	–	+	+	–	+	–	+	+	+
IL–8	+	+	++	+	+	+	+	++	+	+
IL–10	+	+	–	+	++	+	+	+	+	+
MIP–1β	+	+	+	++	++	–	–	+	+	+
Eotaxin–3	–	++	–	–	–	–	+	–	+	–
TARC	+	–	–	–	–	++	–	+	+	–
IP–10	+	+	+	+	+	++	+	+	+	+
IL–8 (HA)	+	+	+	++	+	+	+	+	++	++
MIP–1α	+	+	++	++	++	–	+	–	–	+
MCP–1	+	+	+	+	–	+	+	+	+	+
MDC	+	+	+	+	+	–	+	+	–	+
MCP–4	–	–	+	–	+	+	–	–	+	–

Table 3: Cytokine production upon stimulation

The table below summarizes cross-reactivity for NHP species. For the indicated species, these assays have been used to measure either endogenous analytes in NHP samples (e.g., serum, plasma, urine, or CSF) or analytes produced by cultured NHP cells (e.g., cytokines produced by stimulated PBMCs).

Assay	Cross-Reactive Species
bFGF	Cynomolgus
CA-125	Rhesus, Baboon
CRP	Baboon, Rhesus
Eotaxin	Cynomolgus, Rhesus
EPO	Cynomolgus, Rhesus, Baboon
E-Selectin	Cynomolgus
Flt-1	Cynomolgus
Fractalkine	Cynomolgus
G-CSF	Rhesus
IL-13	Cynomolgus, Rhesus
IL-17D	Cynomolgus
I-TAC	Cynomolgus, Rhesus
MCP-2	Cynomolgus
M-CSF	Cynomolgus
Osteocalcin	Cynomolgus, Rhesus
Osteopontin	Rhesus
Osteoprotegerin	Rhesus
PlGF	Cynomolgus
RANTES	Cynomolgus, Rhesus
Thrombomodulin	Cynomolgus
Tie-2	Cynomolgus
TIMP-1	Rhesus
TNF-RII	Rhesus
TPO	Baboon, Rhesus
TRAIL	Cynomolgus
VEGF-D	Cynomolgus

Table 4: Cross-reactivity of assays with NHP species

These assays may be suitable for primate species other than those indicated due to the high degree of homology among non-human primates.

Assay Components

Antibodies: V-PLEX assays

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
bFGF	Mouse Monoclonal	Mouse Monoclonal	A
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-1β	Mouse Monoclonal	Goat Polyclonal	B
IL-2	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-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
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
PlGF	Mouse Monoclonal	Goat Polyclonal	A
TARC	Mouse Monoclonal	Mouse Monoclonal	B
Tie-2	Mouse Monoclonal	Goat Polyclonal	A
TNF-β	Mouse Monoclonal	Mouse Monoclonal	A
VEGF-A	Mouse Monoclonal	Mouse Monoclonal	C
VEGF-D	Mouse Monoclonal	Goat Polyclonal	A

Table 5: Antibodies for V-PLEX assays

Antibodies: Other Assays

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
CA-125	Mouse Monoclonal	Mouse Monoclonal	A
CRP	Mouse Monoclonal	Mouse Monoclonal	A
Eotaxin	Mouse Monoclonal	Mouse Monoclonal	A
EPO	Mouse Monoclonal	Mouse Monoclonal	A
E-Selectin	Mouse Monoclonal	Mouse Monoclonal	A
Fractalkine	Mouse Monoclonal	Goat Polyclonal	A
G-CSF	Mouse Monoclonal	Goat Polyclonal	A
IL-13	Rat Monoclonal	Goat Polyclonal	A
IL-17D	Mouse Monoclonal	Goat Polyclonal	A
I-TAC	Mouse Monoclonal	Mouse Monoclonal	A
MCP-2	Mouse Monoclonal	Mouse Monoclonal	A
M-CSF	Mouse Monoclonal	Goat Polyclonal	A
Osteocalcin	Mouse Monoclonal	Mouse Monoclonal	A
Osteopontin	Goat Polyclonal	Goat Polyclonal	B
Osteoprotegerin	Mouse Monoclonal	Mouse Monoclonal	A
RANTES	Mouse Monoclonal	Goat Polyclonal	A
Thrombomodulin	Mouse Monoclonal	Mouse Monoclonal	A
TIMP-1	Mouse Monoclonal	Goat Polyclonal	A
TNF-RII	Mouse Monoclonal	Goat Polyclonal	A
TPO	Goat Polyclonal	Goat Polyclonal	A
TRAIL	Mouse Monoclonal	Goat Polyclonal	A

Table 6: Antibodies for selected assays

C of A for Special Order Cytokine Assays (NHP)

The Certificate of Analysis for the Special Order NHP Biomarker Group 1 Kit 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 NHP Biomarker Group 1 Kit contains IFN-γ, IL-2, IL-6, GM-CSF, IL-17A, I-TAC, and Fractalkine. The kit is provided with the Proinflammatory Panel 1 (human) calibrator blend, the Cytokine Panel 1 (human) calibrator blend, and individual calibrators for I-TAC and Fractalkine.

Step 1:

Reconstitute each of the calibrator blends 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 I-TAC and Fractalkine calibrators on wet ice.

Step 2.

Prepare the working stocks. For I-TAC, 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 the 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.

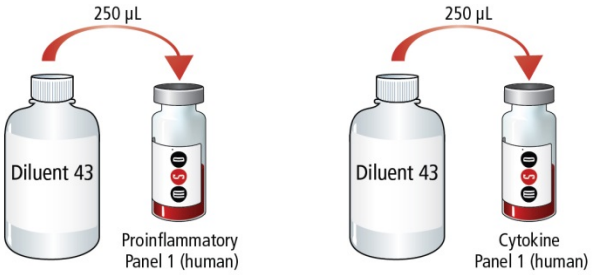
Combine 200 µL of each reconstituted V-PLEX calibrator into one vial. Add 16 µL each of I-TAC and fractalkine working stock to the vial containing the combined reconstituted V-PLEX calibrators. Add 368 µL of Diluent 43 to bring the blended calibrator to a volume of 800 µL. Mix well by vortexing.

After following the instructions above, the blended calibrator concentration should be at the suggested top of curve. The concentration of I-TAC in the vial should be 2,500 pg/mL (see Calibrator 1 concentrations table). The concentration of fractalkine in the vial should be 100,000 pg/mL. The concentrations of IFN-γ, IL-2, IL-6, GM-CSF, and IL-17A are provided in the C of A.

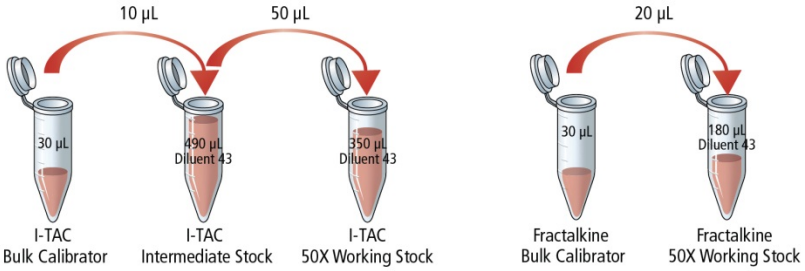
Step 4.

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.

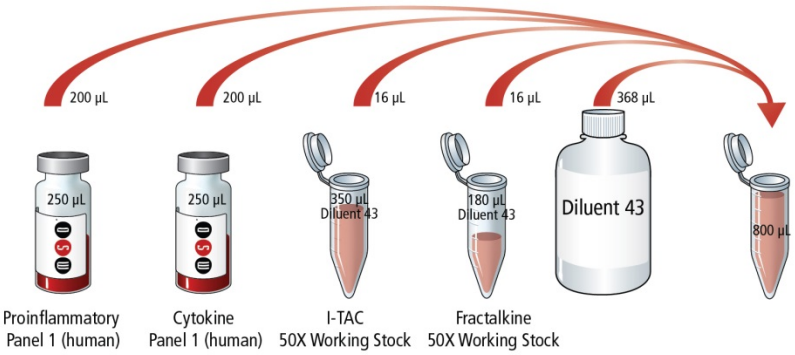
Step 1



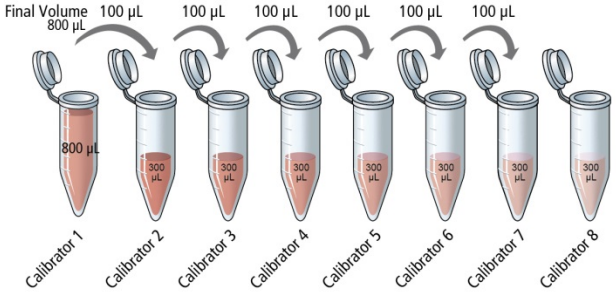
Step 2



Step 3



Step 4



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 higher dilution is suggested below because the assay is present on a panel where other assays require a greater dilution. An example of this is the Chemokine Panel 1 (NHP) 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 suggested 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
bFGF	2		
CA-125	2	2	2
CRP	2		
Eotaxin	2		
Eotaxin-3	4	4	4
EPO	2	2	2
E-Selectin	2	2	
Flt-1	2		
Fractalkine	2		
GM-CSF	2	2	2
G-CSF	2		
IFN-γ	2	2	2
IL-1β	2	2	2
IL-2	2	2	2
IL-5	2	2	2
IL-6	2	2	2
IL-7	2	2	2
IL-8	2	2	2
IL-8 (HA)	4	4	4
IL-10	2	2	2
IL-12/IL-23p40	2	2	2
IL-13	2		
IL-15	2	2	2
IL-16	2	2	2
IL-17A	2	2	2

	Suggested Fold Dilution		
Assay	Serum/Plasma	Urine	CSF
IL-17D	2	2	2
IP-10	4	4	4
I-TAC	2	2	2
MCP-1	4	4	4
MCP-2	4		
MCP-4	4	4	4
M-CSF	2	2	2
MDC	4	4	4
MIP-1α	4	4	4
MIP-1β	4	4	4
Osteocalcin	20		
Osteopontin	2	2	
Osteoprotegerin	50		
PIGF	2		
RANTES	50		
TARC	4	4	4
Thrombomodulin	50		
Tie-2	2		
TIMP-1	50		
TNF-β	2	2	2
TNF-RII	10		
TPO	50		
TRAIL	2	2	
VEGF-A	2	2	2
VEGF-D	2		

Table 7: Suggested sample dilutions