

Fluorokine[®] MAP

Primate Multiplex Kit

Catalog Number LKT006

For the simultaneous quantitative determination of the concentrations of multiple primate biomarkers in serum and EDTA plasma.

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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| 614 McKinley Place NE | | (612) 379-2956 |
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| Abingdon Science Park | E-MAIL: | info@RnDSystems.co.uk |
| Abingdon, OX14 3NB | | |
| United Kingdom | | |

| | | |
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| R&D Systems China Co. Ltd. | TELEPHONE: | +86 (21) 52380373 |
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| 726 West Yan An Road | E-MAIL: | info@RnDSystemsChina.com.cn |
| Shanghai PRC 200050 | | |

INTRODUCTION

Cytokines are secreted signaling molecules that mediate communication between cells. This group of proteins includes interleukins, interferons, and chemokines. Cytokines are secreted by a variety of different cell types and typically act as autocrine or paracrine regulators. They bind to specific receptors on target cells to promote a cascade of intracellular signaling events that regulate numerous physiological and pathological processes including cell growth, differentiation, gene expression, migration, inflammation, and immunity. Their activities can be inhibited by antagonists or neutralizing antibodies that bind either directly to the cytokine itself or to its receptor. Cytokine research is complicated by the fact that these proteins typically have short half-lives and redundant functions. In addition, cytokines commonly operate in large networks, where one cytokine may regulate the production or the activity of other cytokines. For this reason, it is frequently necessary to assess the levels of multiple cytokines present in experimental samples. This process, however, can be tedious and require large sample volumes. Assays that have the capability of simultaneously assessing the levels of numerous cytokines in a single sample offer more efficient approaches for identifying cytokines of interest.

The Fluorokine[®] MAP Primate Multiplex Kit can be used to simultaneously assess the levels of eleven biomarkers in a single sample. For ease of use, the microparticles are pre-mixed in one vial as are the biotinylated detection antibodies.

| Analyte | Bead Region |
|---------------|-------------|
| Eotaxin | 22 |
| IFN- γ | 34 |
| IL-1 β | 6 |
| IL-2 | 17 |
| IL-5 | 9 |
| IL-6 | 32 |
| IL-8 | 36 |
| IL-10 | 50 |
| MCP-1 | 78 |
| MIP-1 β | 74 |
| TNF- α | 77 |

The data cited in this booklet are from Cynomologous monkey samples. Other primate species tested include rhesus macaque, marmoset, capuchin monkey, squirrel monkey, and vervet monkey. Please contact Technical Service for more information.

PRINCIPLE OF THE ASSAY

Fluorokine MAP multiplex kits are designed for use with a Luminex[®] or BioRad[®] BioPlex[®] analyzer, a dual laser, flow-based sorting and detection platform.

Analyte-specific antibodies are pre-coated onto color-coded microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds the biotinylated detection antibodies, is added to each well. A final wash removes unbound Streptavidin-PE and the microparticles are resuspended in buffer and read using the Luminex or BioPlex analyzer. One laser is microparticle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of analyte bound.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, instrumentation, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Fluorokine MAP assay, the possibility of interference cannot be excluded.
- Discrepancies may exist in values obtained for the same analyte utilizing different technologies.
- Fluorokine MAP affords the user the benefit of multianalyte analysis of cytokines in a complex sample. A multipurpose diluent is used to optimize recovery, linearity and reproducibility. Such a multipurpose, single diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the enclosed Standard Value Card can be measured with this kit.**

MATERIALS PROVIDED

Standard Cocktail (Part 893421) - 2 vials of recombinant primate biomarkers in a buffered protein base with preservatives; lyophilized.

Standard Value Card (Part 751684) - 1 card listing the standard reconstitution volume and concentrations for this lot of standard.

Microparticle Cocktail (Part 893403) - 0.60 mL of a concentrated primate microparticle cocktail with preservative.

Biotin Antibody Cocktail (Part 893404) - 0.60 mL of a concentrated primate biotin antibody cocktail with preservatives.

Microparticle Diluent (Part 895529) - 2 vials (6 mL/vial) of a buffered protein base with blue dye and preservative.

Calibrator Diluent RD6-40 (Part 895817) - 21 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

Biotin Antibody Diluent 2 (Part 895832) - 5.5 mL of a buffered protein base with preservative.

Streptavidin-PE (Part 892525) - 0.07 mL of a 100-fold concentrated streptavidin-phycoerythrin conjugate with preservatives.

Microplate (Part 640763) - 1 filter-bottomed plate, used as a vessel for the assay.

Plate Covers (Part 640445) - 6 adhesive foil strips.

PRECAUTION

The Microparticle Cocktail contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

STORAGE

| | | |
|---|---|---|
| Unopened Kit | Store at 2 - 8° C. Do not use past kit expiration date. | |
| Opened/ Reconstituted Reagents | Diluted Wash Buffer | May be stored for up to 1 month at 2 - 8° C. |
| | Biotin Antibody Diluent 2 | |
| | Calibrator Diluent RD6-40 | |
| | Microparticle Diluent | |
| | Streptavidin-PE | |
| | Biotin Antibody Cocktail | |
| | Diluted Biotin Antibody Cocktail | |
| | Microparticle Cocktail | |
| | Diluted Microparticle Cocktail | Discard any unused diluted microparticle mixture. |
| | Standard | Discard after use. Use a fresh standard for each assay. |

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Luminex 100™, Luminex 200™, or BioRad BioPlex Analyzer with X - Y platform.
- Microplate vacuum manifold (Millipore Multiscreen™ Vacuum Manifold, Catalog # MAVM096, or equivalent).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- 15 mL amber conical tubes (Greiner Bio-One, Catalog # 188280 or equivalent) for dilution of microparticles and Streptavidin-PE.
- **Polypropylene** test tubes for dilution.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Microcentrifuge.

SAMPLE COLLECTION AND STORAGE

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and EDTA plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 30 µL of sample + 90 µL of Calibrator Diluent RD6-40. Mix thoroughly.

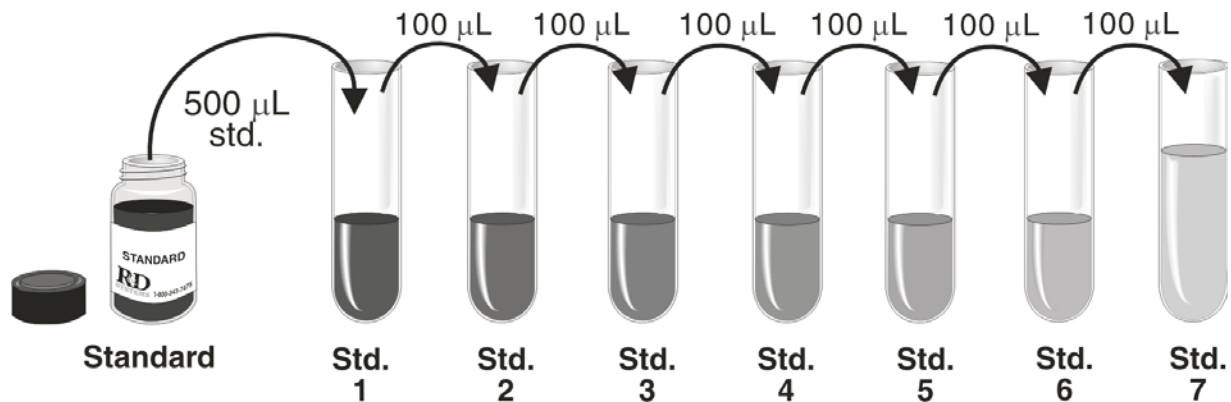
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To assay a full plate, dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer. If assaying a partial plate, prepare only as much Wash Buffer as needed.

Standard - Reconstitute the Standard Cocktail with Calibrator Diluent RD6-40. Refer to the Standard Value Card for the reconstitution volume and assigned values. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 μ L of the reconstituted Standard into the Standard 1 tube. Pipette 200 μ L of Calibrator Diluent RD6-40 into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. Calibrator Diluent RD6-40 serves as the blank.



Diluted Microparticles -

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail in a 15 mL amber conical tube (see the Other Supplies Required section).

| # Wells used | Microparticle Cocktail | + | Microparticle Diluent |
|--------------|------------------------|---|-----------------------|
| 96 | 500 μ L | + | 10.0 mL |
| 72 | 375 μ L | + | 7.5 mL |
| 48 | 250 μ L | + | 5.0 mL |
| 24 | 125 μ L | + | 2.5 mL |

Note: Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.

Diluted Biotin Antibody Cocktail -

1. Centrifuge the Biotin Antibody Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin Antibody Cocktail in Biotin Antibody Diluent. Mix gently.

| # Wells used | Biotin Antibody Cocktail | + | Biotin Antibody Diluent |
|--------------|--------------------------|---|-------------------------|
| 96 | 500 μ L | + | 5.0 mL |
| 72 | 375 μ L | + | 3.75 mL |
| 48 | 250 μ L | + | 2.50 mL |
| 24 | 125 μ L | + | 1.25 mL |

Diluted Streptavidin-PE -

Use a polypropylene amber bottle or a polypropylene tube covered with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the 100X Streptavidin-PE to a 1X concentration by adding 55 μ L of Streptavidin-PE to 5.5 mL of Wash Buffer.

INSTRUMENT SETTINGS

Adjust the probe height setting on the Luminex Analyzer to avoid puncturing the membrane. Refer to the instrument manual.

- a) Assign the bead region for each analyte being measured (refer to page 2)
- b) 50 events/bead
- c) Minimum events: 0
- d) Flow rate: 60 μ L/Min (fast)
- e) Sample size: 50 μ L
- f) Doublet Discriminator gates at approximately 7500 and 15,500
- g) Collect Median RFU

Note: For the BioRad BioPlex analyzer, set the gates at 4300 and 10,000. The CAL2 setting for the BioRad BioPlex analyzer should be set at the Low RP1 target value.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Pre-wet the filter-bottomed microplate by filling each well with 100 μL of Wash Buffer. Remove the liquid through the filter at the bottom of the plate using a vacuum manifold designed to accommodate a microplate.
3. Add 50 μL of Standard or sample* per well.
4. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 100 μL of the diluted Microparticle Cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 2.5 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Using a vacuum manifold device designed to accommodate a microplate, wash by removing the liquid, filling each well with Wash Buffer (100 μL) and removing the liquid again. All of the liquid must be removed through the filter at the bottom of the plate to avoid any loss of microparticles. Complete removal of liquid is essential for good performance. Perform the wash procedure three times.

Note: *After each final wash cycle and subsequent reagent addition, blot the bottom of the plate with a paper towel to prevent wicking.*

6. Add 50 μL of the diluted Biotin Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 500 ± 50 rpm.
7. Repeat the wash as in step 5.
8. Add 50 μL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 500 ± 50 rpm.
9. Repeat the wash as in step 5.
10. Resuspend the microparticles by adding 100 μL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 500 ± 50 rpm.
11. Read within 90 minutes using the Luminex or BioRad Analyzer.

*Samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Pre-wet the plate by filling each well with 100 μ L of Wash Buffer. Remove the liquid through the filter at the bottom of the plate using a vacuum manifold designed to accommodate a microplate.



3. Add 50 μ L of Standard or sample* to each well.



4. Add 100 μ L of diluted Microparticle Cocktail to each well. Incubate 2.5 hours at RT on a horizontal orbital microplate shaker.



5. Wash by removing the liquid from each well, filling each well with Wash Buffer (100 μ L), and removing the liquid again. Perform the wash 3 times.



6. Add 50 μ L of diluted Biotin Antibody Cocktail to all wells. Incubate 1 hour at RT on the shaker.



7. Repeat the wash as in step 5.



8. Add 50 μ L of diluted Streptavidin-PE to all wells. Incubate 30 minutes at RT on the shaker.



9. Repeat the wash as in step 5.



10. Add 100 μ L of Wash Buffer to each well. Incubate 2 minutes at RT on the shaker.



11. Read within 90 minutes using the Luminex or BioRad Analyzer. Refer to the Instrument Settings section.

Protect microparticles and Streptavidin-PE from light at all times.

*Samples require dilution. See Sample Preparation.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.
- For best results, adjust the vacuum strength to between 15 and 40 cm of mercury.

SENSITIVITY

Twenty-six assays were evaluated. The minimum detectable dose of each biomarker in this panel was determined by adding two standard deviations to the mean RFU of twenty zero standard replicates and calculating the corresponding concentration.

| Analytes | Mean (pg/mL) | Range (pg/mL) |
|---------------|--------------|---------------|
| Eotaxin | 1.76 | 0.98 - 4.28 |
| IFN- γ | 0.26 | 0.14 - 0.66 |
| IL-1 β | 0.41 | 0.20 - 0.81 |
| IL-2 | 2.22 | 1.19 - 3.33 |
| IL-5 | 0.27 | 0.09 - 0.54 |
| IL-6 | 1.77 | 0.67 - 6.84 |
| IL-8 | 0.42 | 0.20 - 1.10 |
| IL-10 | 0.31 | 0.18 - 0.93 |
| MCP-1 | 1.50 | 0.85 - 3.16 |
| MIP-1 β | 3.49 | 1.47 - 10.58 |
| TNF- α | 2.74 | 1.32 - 7.54 |

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank median RFU.

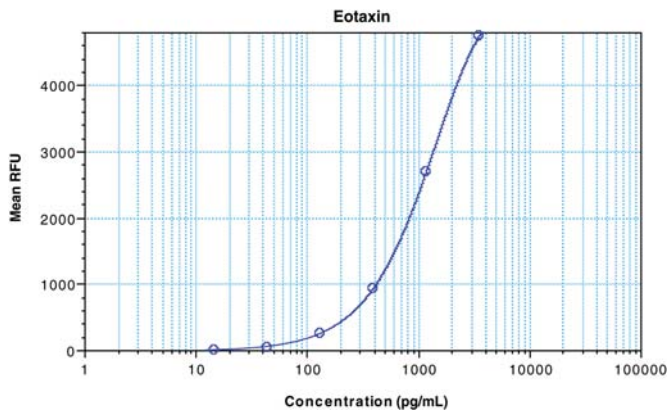
Create a standard curve for each analyte by reducing the data using computer software capable of generating a 5-PL curve fit. As an alternative, construct a standard curve by plotting the median RFU for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the cytokine concentrations versus the log of the RFU and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

To determine the cytokine concentration of each sample, first find the RFU value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding cytokine concentration.

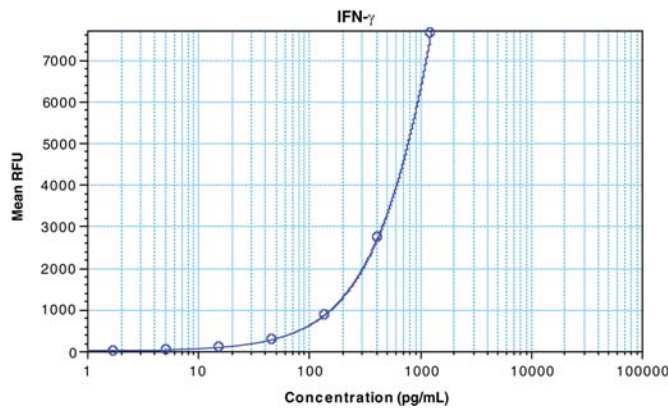
Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

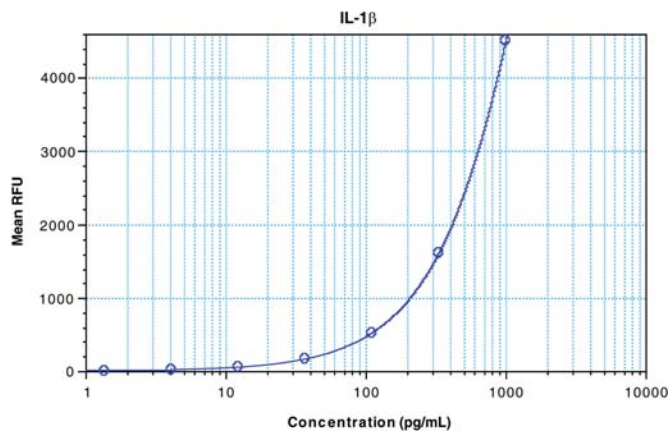
These standard curves are provided only for demonstration. Standard curves must be generated each time an assay is run, using the values from the included Standard Value Card.



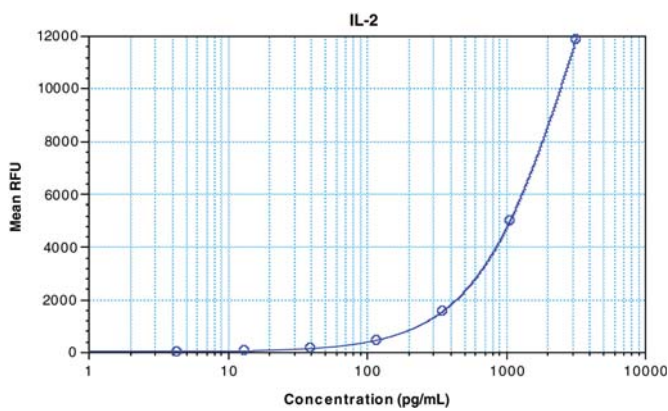
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|----------------------|---------|-----------|
| Blank | 0 | 4 | 4 | — |
| 1 | — | — | — | — |
| 2 | 3530 | 4727 4756 2570 | 4742 | 4738 |
| 3 | 1180 | 2815 | 2692 | 2688 |
| 4 | 393 | 902 961 258 | 932 | 928 |
| 5 | 131 | 261 52 | 260 | 256 |
| 6 | 44 | 52 12 | 52 | 48 |
| 7 | 15 | 14 | 13 | 9 |



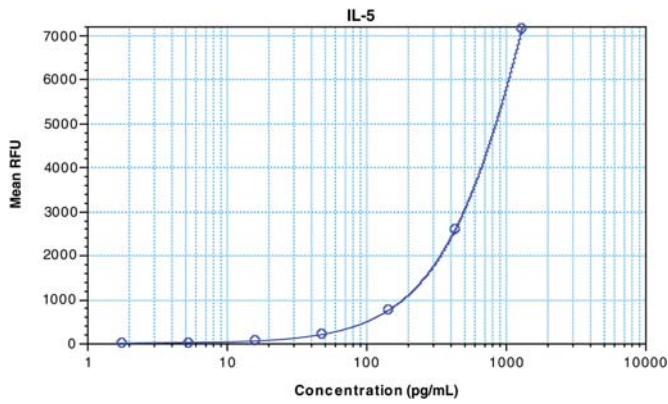
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|----------------------|---------|-----------|
| Blank | 0 | 2 3 | 3 | — |
| 1 | 1250 | 7510 7743 2687 | 7626 | 7623 |
| 2 | 417 | 2781 | 2734 | 2731 |
| 3 | 139 | 836 914 263 | 875 | 872 |
| 4 | 46 | 296 96 | 280 | 277 |
| 5 | 15 | 96 32 | 96 | 93 |
| 6 | 5.1 | 33 12 | 33 | 30 |
| 7 | 1.7 | 13 | 13 | 10 |



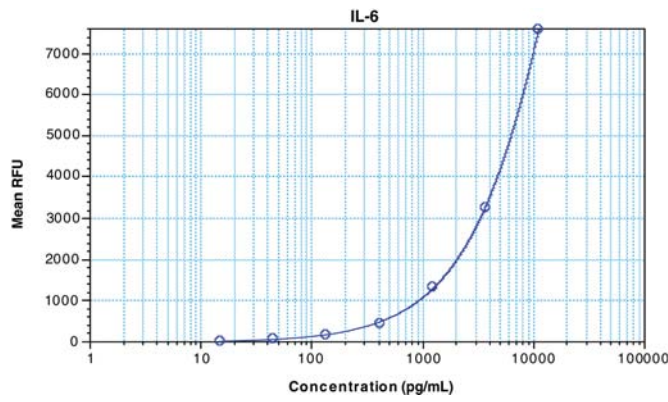
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|----------------------|---------|-----------|
| Blank | 0 | 2 2 | 2 | — |
| 1 | 1000 | 4436 4602 1603 | 4519 | 4517 |
| 2 | 333 | 1635 517 | 1619 | 1617 |
| 3 | 111 | 521 165 | 519 | 517 |
| 4 | 37 | 187 57 | 176 | 174 |
| 5 | 12 | 59 17 | 58 | 56 |
| 6 | 4.1 | 20 6.5 | 19 | 17 |
| 7 | 1.4 | 8.0 | 7.3 | 5.3 |



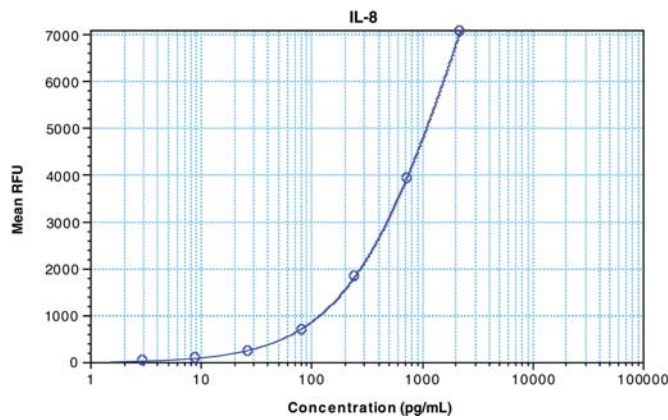
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|------------------------|---------|-----------|
| Blank | 0 | 41.5 52.5 11,239 | 47 | — |
| 1 | 3200 | 12,581 4831 | 11,910 | 11,863 |
| 2 | 1070 | 5191 1534 | 5011 | 4964 |
| 3 | 357 | 1699 478 | 1616 | 1569 |
| 4 | 119 | 479 184 | 479 | 432 |
| 5 | 40 | 189 91 | 187 | 140 |
| 6 | 13 | 92 61 | 92 | 45 |
| 7 | 4.4 | 62 | 62 | 15 |



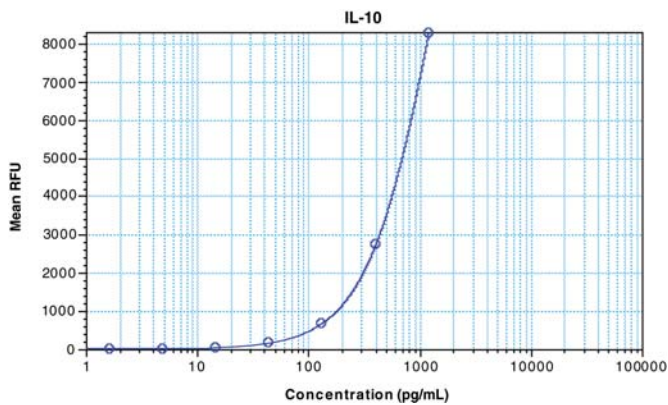
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|----------------|---------|-----------|
| Blank | 0 | 2 3 7035 | 2 | — |
| 1 | 1300 | 7232 2542 | 7133 | 7131 |
| 2 | 433 | 2637 742 | 2590 | 2588 |
| 3 | 144 | 776 186 | 759 | 757 |
| 4 | 48 | 198 51 | 192 | 190 |
| 5 | 16 | 53 15 | 52 | 50 |
| 6 | 5.3 | 15 6 | 15 | 13 |
| 7 | 1.8 | 6 | 6 | 4 |



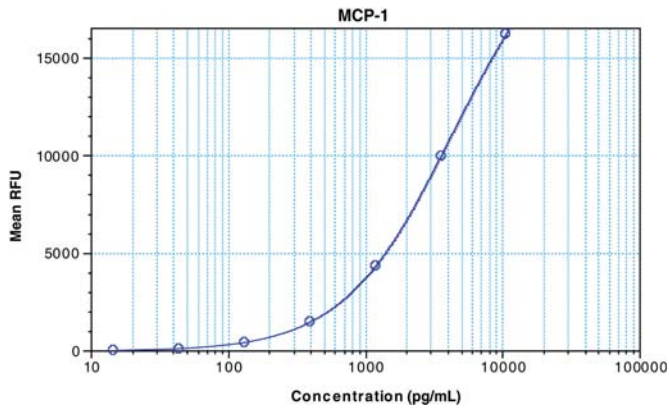
| Standard | pg/mL | RFU | Average | Corrected |
|----------|--------|----------------|---------|-----------|
| Blank | 0 | 4 5 7422 | 4 | — |
| 1 | 11,100 | 7707 3126 | 7565 | 7561 |
| 2 | 3700 | 3402 1290 | 3264 | 3260 |
| 3 | 1230 | 1333 444 | 1312 | 1308 |
| 4 | 410 | 444 145 | 444 | 440 |
| 5 | 137 | 163 50 | 154 | 150 |
| 6 | 46 | 51 19 | 51 | 47 |
| 7 | 15 | 19 | 19 | 15 |



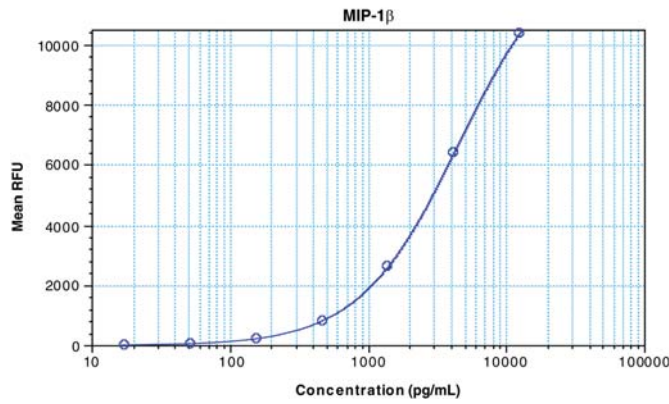
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|------------------|---------|-----------|
| Blank | 0 | 25 25 6949 | 25 | — |
| 1 | 2200 | 7220 3914 | 7085 | 7060 |
| 2 | 733 | 4006 1810 | 3960 | 3935 |
| 3 | 244 | 1908 698 | 1859 | 1834 |
| 4 | 81.3 | 753 255 | 726 | 701 |
| 5 | 27.1 | 261 102 | 258 | 233 |
| 6 | 9.0 | 110 51 | 106 | 81 |
| 7 | 3.0 | 53 | 52 | 27 |



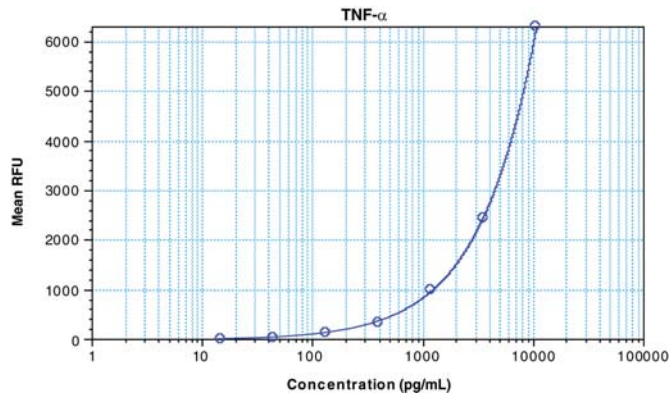
| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|----------------|---------|-----------|
| Blank | 0 | 5 5 7813 | 5 | — |
| 1 | 1200 | 8751 2725 | 8282 | 8277 |
| 2 | 400 | 2745 652 | 2735 | 2730 |
| 3 | 133 | 714 157 | 683 | 678 |
| 4 | 44 | 177 47 | 167 | 162 |
| 5 | 15 | 53 16 | 50 | 45 |
| 6 | 4.9 | 16 8 | 16 | 11 |
| 7 | 1.6 | 9 | 8.5 | 3.5 |



| Standard | pg/mL | RFU | Average | Corrected |
|----------|-------|------------------------|---------|-----------|
| Blank | 0 | 34.5 41.5 15,937 | 38 | — |
| 1 | 4400 | 16,500 9628 | 16,219 | 16,181 |
| 2 | 1470 | 10,270 4226 | 9949 | 9911 |
| 3 | 490 | 4463 1458 | 4344 | 4306 |
| 4 | 163 | 1517 425 | 1488 | 1450 |
| 5 | 54 | 474 113 | 449 | 411 |
| 6 | 18 | 117 48 | 115 | 77 |
| 7 | 6 | 52 | 50 | 12 |



| Standard | pg/mL | RFU | Average | Corrected |
|----------|--------|--------------------|---------|-----------|
| Blank | 0 | 12 13 10,322 | 12 | — |
| 1 | 12,600 | 10,477 6357 | 10,400 | 10,388 |
| 2 | 4200 | 6457 2594 | 6407 | 6395 |
| 3 | 1400 | 2662 815 | 2628 | 2616 |
| 4 | 467 | 853 209 | 834 | 822 |
| 5 | 156 | 212 56 | 210 | 198 |
| 6 | 52 | 57 25 | 57 | 45 |
| 7 | 17 | 26 | 26 | 14 |



| Standard | pg/mL | RFU | Average | Corrected |
|----------|--------|------------------|---------|-----------|
| Blank | 0 | 10 10 6230 | 10 | — |
| 1 | 10,700 | 6371 2441 | 6300 | 6290 |
| 2 | 3570 | 2463 994 | 2452 | 2442 |
| 3 | 1190 | 1023 350 | 1008 | 998 |
| 4 | 397 | 355 126 | 352 | 342 |
| 5 | 132 | 131 46 | 129 | 119 |
| 6 | 44 | 49 21 | 48 | 38 |
| 7 | 15 | 22 | 21 | 11 |

PRECISION

Intra-assay Precision (Precision within an assay)

Samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Samples of known concentration were tested in sixty-seven separate assays to assess inter-assay precision.

Eotaxin Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|------|------|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 44.6 | 245 | 1802 | 46.8 | 42.5 | 244 | 1985 | 50.1 |
| Standard Deviation | 1.3 | 11.1 | 161 | 2.7 | 4.5 | 22.8 | 315 | 5.4 |
| CV (%) | 2.9 | 4.5 | 8.9 | 5.8 | 10.5 | 9.3 | 15.9 | 10.8 |

IFN- γ Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|------|-----|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 9.0 | 42.8 | 328 | 6.6 | 8.8 | 45.6 | 321 | 6.9 |
| Standard Deviation | 0.6 | 2.0 | 12.1 | 0.3 | 0.8 | 4.0 | 22.7 | 0.7 |
| CV (%) | 6.8 | 4.6 | 3.7 | 4.1 | 9.3 | 8.8 | 7.1 | 10.1 |

IL-1 β Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|-----|-----|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 7.9 | 35.7 | 267 | 5.6 | 7.4 | 35.7 | 259 | 5.3 |
| Standard Deviation | 0.5 | 1.9 | 16 | 0.4 | 1.0 | 3.3 | 14.7 | 0.7 |
| CV (%) | 6.7 | 5.3 | 5.9 | 7.5 | 14.2 | 9.3 | 5.7 | 14.1 |

IL-2 Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|-----|------|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 19.7 | 79.1 | 610 | 14.2 | 15.5 | 83.3 | 608 | 15.9 |
| Standard Deviation | 1.4 | 3.6 | 37 | 1.4 | 2.4 | 9.2 | 47.7 | 2.2 |
| CV (%) | 7.1 | 4.6 | 6.0 | 9.9 | 15.5 | 11.0 | 7.8 | 13.7 |

IL-5 Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|-----|-----|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 9.6 | 41.7 | 321 | 6.6 | 8.8 | 45.9 | 330 | 7.3 |
| Standard Deviation | 0.5 | 2.3 | 12 | 0.4 | 0.8 | 4.2 | 21.9 | 0.7 |
| CV (%) | 4.7 | 5.5 | 3.8 | 6.2 | 8.9 | 9.1 | 6.6 | 10.2 |

IL-6 Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|------|------|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 74.9 | 351 | 2629 | 58.4 | 71.1 | 367 | 2642 | 60.3 |
| Standard Deviation | 2.8 | 14.5 | 169 | 2.9 | 6.2 | 32.6 | 209 | 5.6 |
| CV (%) | 3.8 | 4.1 | 6.4 | 4.9 | 8.8 | 8.9 | 7.9 | 9.2 |

IL-8 Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|-----|-----|------|-----------------------|-----|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 21.7 | 102 | 682 | 12.1 | 19.9 | 100 | 738 | 12.5 |
| Standard Deviation | 1.0 | 6.1 | 60 | 0.7 | 1.8 | 8.2 | 67.3 | 1.2 |
| CV (%) | 4.7 | 5.9 | 8.7 | 6.0 | 9.1 | 8.2 | 9.1 | 10.0 |

IL-10 Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|-----|-----|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 8.0 | 37.2 | 279 | 6.7 | 7.6 | 38.8 | 271 | 7.4 |
| Standard Deviation | 0.2 | 1.3 | 14 | 0.5 | 0.8 | 3.8 | 16.5 | 0.8 |
| CV (%) | 2.9 | 3.6 | 5.1 | 7.3 | 10.5 | 9.8 | 6.1 | 10.6 |

MCP-1 Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|-----|------|------|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 36.3 | 197 | 1493 | 18.5 | 34.4 | 202 | 1651 | 19.5 |
| Standard Deviation | 1.3 | 9.5 | 131 | 0.9 | 3.2 | 21.6 | 168 | 2.4 |
| CV (%) | 3.6 | 4.8 | 8.8 | 5.1 | 9.4 | 10.7 | 10.1 | 12.2 |

MIP-1 β Assay

| Sample | Intra-assay Precision | | | | Inter-assay Precision | | | |
|--------------------|-----------------------|------|------|------|-----------------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| n | 20 | 20 | 20 | 20 | 67 | 67 | 67 | 67 |
| Mean (pg/mL) | 65.5 | 336 | 2873 | 66.8 | 57.1 | 323 | 2723 | 64 |
| Standard Deviation | 2.2 | 13.8 | 182 | 2.9 | 6.5 | 29.0 | 314 | 6.6 |
| CV (%) | 3.3 | 4.1 | 6.3 | 4.4 | 11.3 | 9.0 | 11.5 | 10.4 |

TNF- α Assay

| Sample | Intra-assay Precision | | Inter-assay Precision | |
|--------------------|-----------------------|------|-----------------------|------|
| | 1 | 2 | 1 | 2 |
| n | 20 | 20 | 67 | 67 |
| Mean (pg/mL) | 16.9 | 85.1 | 82.9 | 762 |
| Standard Deviation | 1.4 | 3.9 | 14.1 | 101 |
| CV (%) | 8.2 | 4.5 | 17.0 | 13.3 |

RECOVERY

The recovery of the analytes included in this panel was evaluated. Recombinant proteins were spiked to levels throughout the range of the assay in serum matrix and tested as a multiplex.

| Analyte | Average % Recovery | Range |
|---------------|--------------------|------------|
| Eotaxin | 104 | 94 - 115% |
| IFN- γ | 104 | 90 - 116% |
| IL-1 β | 101 | 75 - 112% |
| IL-2 | 88 | 75 - 99% |
| IL-5 | 105 | 94 - 112% |
| IL-6 | 106 | 86 - 119% |
| IL-8 | 111 | 94 - 125% |
| IL-10 | 107 | 100 - 115% |
| MCP-1 | 104 | 90 - 113% |
| MIP-1 β | 113 | 104 - 121% |
| TNF- α | 102 | 97 - 107% |

LINEARITY

To assess the linearity of the assay, serum samples containing and/or spiked with high concentrations of the analytes in this panel were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

| | | Eotaxin | IFN- γ | IL-1 β | IL-2 | IL-5 | IL-6 |
|------|-----------------------|-----------|---------------|--------------|----------|----------|----------|
| 1:2 | Average % of Expected | 110 | 105 | 103 | 99 | 101 | 102 |
| | Range (%) | 93 - 140 | 102 - 107 | 100 - 108 | 88 - 108 | 97 - 104 | 97 - 109 |
| 1:4 | Average % of Expected | 107 | 101 | 105 | 99 | 93 | 95 |
| | Range (%) | 100 - 123 | 92 - 107 | 95 - 109 | 85 - 113 | 89 - 98 | 83 - 100 |
| 1:8 | Average % of Expected | 105 | 99 | 105 | 98 | 91 | 93 |
| | Range (%) | 95 - 109 | 86 - 106 | 101 - 109 | 83 - 114 | 86 - 98 | 88 - 102 |
| 1:16 | Average % of Expected | 102 | 97 | 105 | 93 | 88 | 92 |
| | Range (%) | 90 - 108 | 89 - 104 | 98 - 111 | 71 - 115 | 84 - 90 | 88 - 98 |

| | | IL-8 | IL-10 | MCP-1 | MIP-1 β | TNF- α |
|------|-----------------------|----------|----------|----------|---------------|---------------|
| 1:2 | Average % of Expected | 103 | 102 | 101 | 95 | 111 |
| | Range (%) | 91 - 116 | 99 - 108 | 90 - 111 | 79 - 112 | 99 - 122 |
| 1:4 | Average % of Expected | 100 | 95 | 96 | 89 | 107 |
| | Range (%) | 91 - 125 | 90 - 103 | 87 - 107 | 69 - 110 | 96 - 118 |
| 1:8 | Average % of Expected | 103 | 98 | 97 | 88 | 109 |
| | Range (%) | 94 - 120 | 89 - 110 | 91 - 109 | 68 - 115 | 97 - 116 |
| 1:16 | Average % of Expected | 106 | 95 | 98 | 86 | 105 |
| | Range (%) | 95 - 127 | 84 - 110 | 94 - 105 | 69 - 108 | 91 - 120 |

SPECIFICITY

This assay recognizes both natural and recombinant primate proteins. The following factors were assayed at 50 ng/mL for cross-reactivity in this Fluorokine MAP Primate Multiplex Kit. No cross-reactivity was observed with members of the multiplex panel.

Recombinant human:

| | | | |
|--------------|---------------|------------------|----------------|
| 6Ckine | I-309 | IL-19 | MIG |
| Amphiregulin | IFN- γ | IL-20 | MIP-1 α |
| Angiogenin | IL-1 α | IL-20 R α | MIP-1 β |
| BDNF | IL-1 β | IL-20 R β | MIP-1 δ |
| BLC/BCA | IL-1ra | IL-22 | MIP-3 α |
| CD4 | IL-2 | IL-24 | MIP-3 β |
| CD40 Ligand | IL-3 | IL-26 | NAP-2 |
| CNTF | IL-4 | IL-26 (dimer) | NT-4 |
| CTACK | IL-5 | IL-26 (monomer) | OPG |
| CTLA-4 | IL-6 | IL-28A | OSM |
| ENA-78 | IL-7 | IL-29 | SCF |
| Eotaxin-2 | IL-8 | IP-10 | SDF-1 α |
| Eotaxin-3 | IL-9 | I-TAC | SDF-1 β |
| Fas | IL-10 | Leptin | TARC |
| GCP-2 | IL-11 | LIF | TNF- α |
| GDNF | IL-12 | MCP-1 | TNF- β |
| GITR Ligand | IL-12 p40 | MCP-2 | Tpo |
| GRO α | IL-13 | MCP-3 | TRAIL |
| GRO β | IL-15 | MCP-4 | TECK |
| GRO γ | IL-16 | MDC | |
| HCC-4 | IL-17 | MIF | |

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