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Technical Note

Use of MILLIPLEX® MAP Human IL-18 Singleplex Kit

Introduction

Interleukin-18 (IL-18, also known as interferon-gamma inducing factor) is a member of the IL-1 cytokine superfamily and acts as a pleiotropic cytokine that modulates both innate and acquired immune responses. Disrupted regulation of IL-18 bioactivity is linked to multiple types of diseases including chronic inflammatory diseases, autoimmune diseases, a variety of cancers, and infectious diseases. It also has been implicated to play a role in emphysema, myocardial function, cardiovascular disease, metabolic syndrome, acute kidney injury, and neurodegeneration. Since this protein is being investigated in many research areas, we have provided it as a single protein assay using Luminex® xMAP® technology, in the form of a MILLIPLEX® MAP Human IL-18 Singleplex Magnetic Bead Kit (Catalog No. HIL18MAG-66K). As a single assay, this kit is calibrated to the WHO standard for IL-18 (NIBSC Code 03/200, was from NIBSC, Hertsfordshire, UK).

Our MILLIPLEX® MAP Human IL-18 Singleplex Magnetic Bead Kit is a single protein kit to be used for the quantification of IL-18 in serum, plasma, and tissue/cell supernatant samples using Luminex® xMAP® technology. Additionally, this kit can be combined and assayed within the following kits:

- MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel I, Catalog No. HCYTOMAG-60K (or HCYTMAG-60K-PX29, HCYTMAG-60K-PX30, HCYTMAG-60K-PX38, HCYTMAG-60K-PX41, or the corresponding bulk format kits)
- MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel II, Catalog No. HCYP2MAG-62K (except for the CTACK/CCL27 assay, due to bead region overlap)
- MILLIPLEX® MAP Human Th17 Magnetic Bead Panel, Catalog No. HTH17MAG-14K (or HT17MG-14K-PX25, or the corresponding bulk format kit)

Methods

Human IL-18 as a single protein assay

It is straightforward to run this kit as a single protein assay. Simply use the reagents that come in the kit, and follow the manual provided with the kit from reagent preparations (in the section of the protocol entitled “Preparation of Reagents for Immunoassay: Single Human IL-18 Analyte Only”), and skipping to the immunoassay procedure (in the section of the protocol entitled “Immunoassay Procedure: Single Human IL-18 Analyte Only”). As always, the entire manual should be read and fully understood before running the assay.

Figure 1 shows an example of a typical standard curve obtained by running this assay, along with the locations of the Quality Controls on the curve. **Table 1** shows the standard curve data, sensitivity and precision obtained when running this assay.

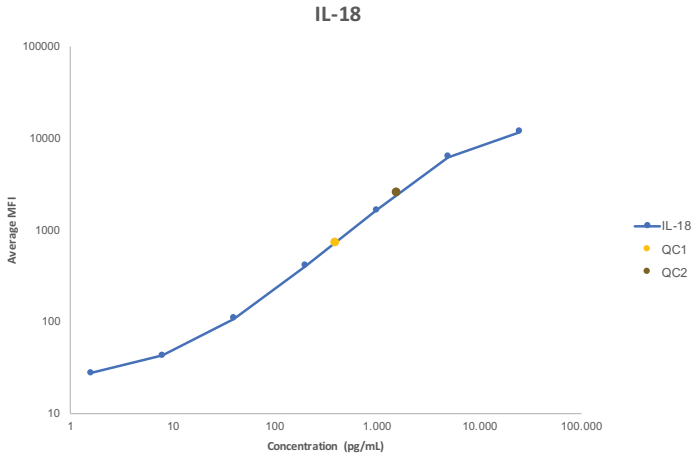

Figure 1. Representative standard curve of the Human IL-18 Singleplex Kit.

(A)

	IL-18 (pg/mL)	IL-18 (MFI)
Standard 1	2	27
Standard 2	8	42
Standard 3	40	108
Standard 4	200	407
Standard 5	1,000	1,635
Standard 6	5,000	6,251
Standard 7	25,000	11,664

(B)

		IL-18
Sensitivity[1]		1.5 pg/mL
Intra-assay CV (%)[2]	Quality Control 1	3.2
	Quality Control 2	6.0
Inter-assay CV (%)[3]	Quality Control 1	4.6
	Quality Control 2	4.5

[1] **n = 11** [2] **n = 8** [3] **n = 7**

Table 1. (A) Representative standard curve data, shown in pg/mL and in Median Fluorescence Intensity (MFI) for the kit when run as singleplex. **(B)** Sensitivity (in pg/mL) and precision (in % CV) for the kit when run as singleplex.

Human IL-18 combined with another kit

When combining the IL-18 analyte with one of the larger previously indicated panels, use the indicated reagents provided in the other kit. Carefully reference the kit manual for both kits to ensure proper procedures are followed, particularly for the following sections:

- "Preparation of Reagents for Immunoassay: Human IL-18 with Other Kits."
  Note: use the serum matrix (if needed) provided with the larger panel.
- Follow the "Immunoassay Procedure" for the larger panel.

There are four main steps in combining the MILLIPLEX® MAP Human IL-18 Singleplex Kit with the other indicated kits:

1. Add an aliquot of the Human IL-18 Beads (HIL18-MAG) to the mixture of beads provided in the other kit (as indicated in the manual for HIL18MAG-66K).
2. Add an aliquot of the Human IL-18 Detection Antibody (HIL18-1066) to the Detection Antibody Cocktail provided in the other kit.
3. Reconstitute the concentrated Human IL-18 Quality Controls 1 and 2 (HIL18-6066) in water, dilute to their final concentrations, and use these dilutions to reconstitute the Quality Controls 1 and 2 provided in the other kit.
4. Reconstitute the Human IL-18 Standard (HIL18-8066) in water, dilute to the final concentration, and use the dilution to reconstitute the Standard provided in the other kit. Follow the Standard dilution protocol of the other kit.

After combining in the Human IL-18 reagents, refer to the assay protocol of the other kit.

Table 2 shows the working standard curve concentrations when combined into the indicated kits.

HIL-18 in HCYTOMAG-60K (pg/mL)	HIL-18 in HCYP2MAG-62K (pg/mL)	HIL-18 in HTH17MAG-14K (pg/mL)
25,000	5,000	25,000
5,000	1,250	6,250
1,000	312.5	1,563
200	78.1	390.6
40	19.5	97.7
8	4.9	24.4
--	--	6.1

Table 2. Human IL-18 will have the resulting standard curve concentrations in the kits indicated, as shown. For use with HCYP2MAG-62K, please note the CTACK/CCL27 assay may not be run together with the IL-18 assay due to bead region overlap.

Human IL-18 combined with HCYTOMAG-60K

To test combining the singleplex kit with a larger panel, the MILLIPLEX® MAP Human IL-18 Singleplex Kit was combined with the Human Cytokine/Chemokine Panel I (Catalog No. HCYTOMAG-60K) as described in the protocol for HIL18MAG-66K, and moving to the protocol for HCYTOMAG-60K where indicated. The multiplex assay was performed in a 96-well plate. The detailed procedure is as follows:

1. Wet the plate with 200 µL wash buffer for 10 min and decant.
2. Add 25 µL standards or samples, 25 µL beads, and 25 µL appropriate matrix solution or assay buffer and incubate overnight at 4°C.
3. Wash the beads three times then add 25 µL biotinylated detection antibody cocktail and incubate at RT for 1 hour.
4. Add 25 µL Streptavidin-Phycoerythrin and further incubate at RT for 30 min.
5. Wash beads three times, add 150 µL sheath fluid and read on Luminex® instrumentation.

Serum and plasma samples were tested. Sepsis samples were obtained from Discovery Life Sciences Inc., Los Osos, CA. Normal control samples were obtained from Bioreclamation LLC, Westbury, NY. **Figure 2** shows example results when combining the IL-18 assay into the larger Human Cytokine/Chemokine Panel I.

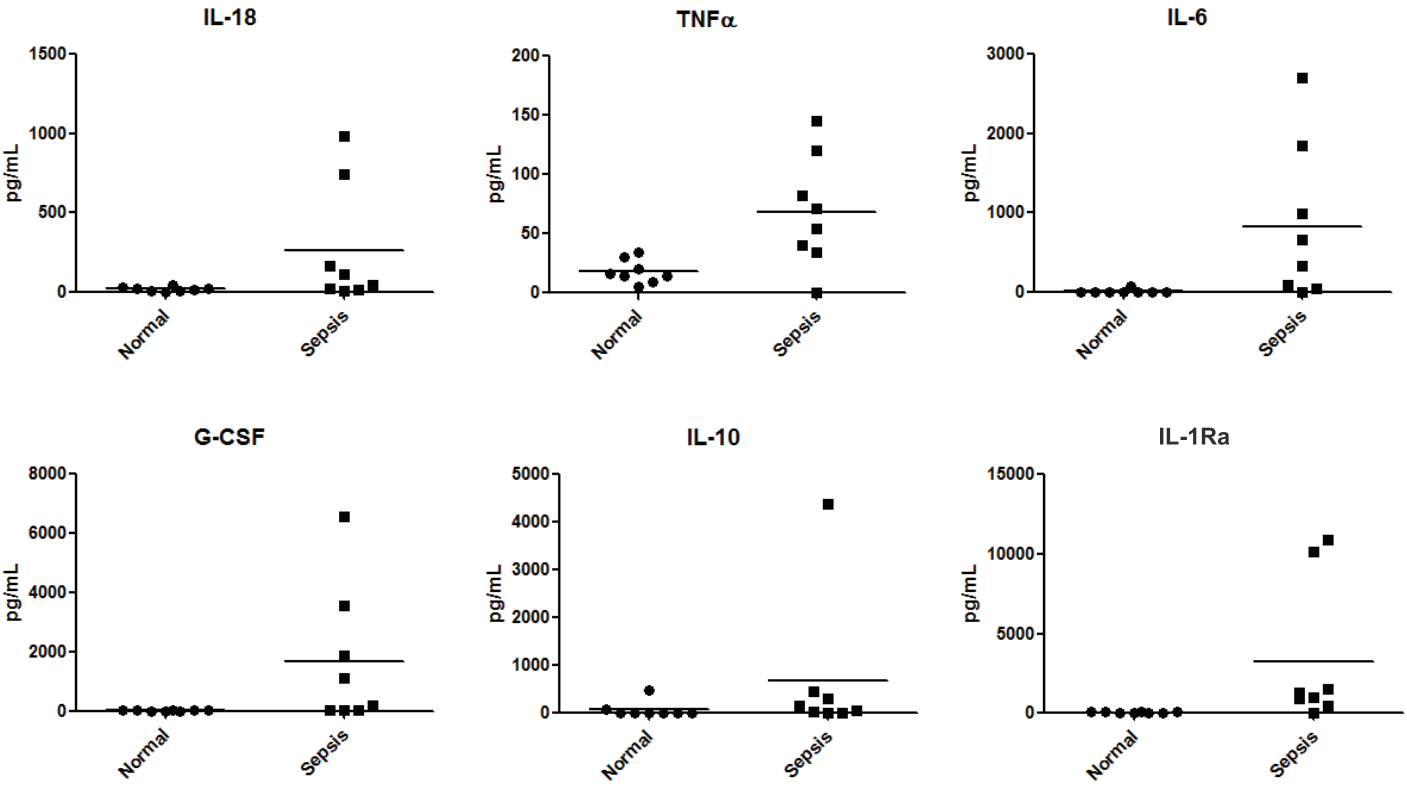

Figure 2. Example results using serum and plasma samples from normal control samples and sepsis samples when combining the MILLIPLEX® MAP Human IL-18 Singleplex Kit with the Human Cytokine/Chemokine Panel I. Mean values are indicated. Normal samples, n= 8; Sepsis samples n=8.

Conclusion

The MILLIPLEX® MAP Human IL-18 Singleplex Kit is a versatile tool for the researcher who works with quantitative immunoassays. As a single assay, this kit is calibrated to the WHO standard for IL-18.

In addition, this singleplex assay may be combined with other kits in the portfolio, using specifically outlined protocols for three of our human cytokine kits. It is possible to combine this assay with additional kits; however, please contact our Technical Support for details. IL-18 sample values observed when combining the Human IL-18 Singleplex Kit into other kits may change depending on the serum matrix added to the standard curves.

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