

Procarta[®] Canine Cytokine Assay Kit

Multiplexed quantification of canine cytokines

Current methods for the detection and quantitation of cytokines, including enzyme-linked immunosorbent assays (ELISA), flow cytometry, western blotting, and protein arrays, are limited by complexity, high sample volume requirements, sensitivity concerns, throughput, and/or quantitative abilities.

The Procarta[®] Canine Cytokine Assay Kit from Affymetrix is a simple, precise, and sensitive method for the measurement of cytokines in a multiplex format. This Luminex[®] bead-based assay utilizes xMAP[®] technology for simultaneous quantitation of protein cytokines from serum, plasma, tissue lysates, and cell culture supernatant samples.

Benefits include:

- Simple workflow
- Cost efficiency
- Less-intensive labor

The kit enables you to:

- Process larger sample batches in less time
- Obtain a wider range of quantitative data with minimal sample volume
- Simultaneously detect up to 12 canine cytokines in a single, three-hour reaction

Introduction

Cytokines are secreted proteins that play a key role in innate immunity, apoptosis, angiogenesis, cell growth, and differentiation. These proteins have also been implicated in disease processes, including infection, inflammation, cancer, and cardiac diseases. The interaction between cytokines and the cellular immune system is a dynamic process that involves interplay between positive and negative stimuli, as well as positive and negative regulatory loops. Furthermore, multiple cytokines are usually involved in a given process.

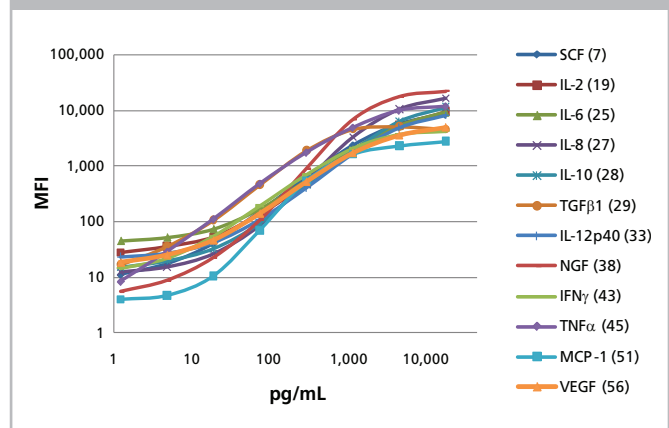
The Procarta Canine Cytokine Assay Kit enables the simultaneous detection of up to 12 cytokines from canine serum, plasma, cell lysates, or cell culture supernatant samples. Capture antibodies that specifically recognize the target cytokine are

conjugated onto fluorescent beads. Each target protein binds to a specific capture bead and the captured target protein is labeled by a biotinylated antibody together with streptavidin-PE (SAPE) dye. The fluorescent signal emitted from each labeled bead can be detected using a Luminex-based instrument. The assay is highly sensitive and quantitative, and can be completed in less than three hours.

Detection range and sensitivity

Figure 1 shows typical standard curves for the Procarta Canine Cytokine Assay Kit. The standard curves for 12 targets ranged from 1.22 to 20,000 pg/mL for serum/plasma samples in the assay well. The typical limit of detection was between 0.7 and 7.6 pg/mL, as shown in Table 1 (p. 2).

Figure 1: Typical standard curves for the Procarta Canine Cytokine Assay Kit. Premixed lyophilized antigen standard provided in the kit contains recombinant cytokines in the amount indicated in the instructions. The premixed standard was reconstituted in assay buffer and four-fold serial dilutions were prepared. Each diluted standard was assayed in duplicate.



Precision

To measure intra- and inter-assay precision, samples of known concentration were tested 20 times on one plate, and five plates were processed (Table 1). All data were processed as a multiplex. The average coefficient of variation (CV) for intra-assay precision was 5.4 percent and for inter-assay precision was 10.5 percent.

To test the recovery of the assay, recombinant cytokines were spiked in and the samples were assayed in multiplex format. The typical recovery range per target is shown in Table 1.

Detecting secreted cytokines from a canine macrophage cell line

A canine macrophage cell line, DH82, treated with lipopolysaccharide (LPS), was used as a model for secretion of pro-inflammatory cytokines under immune response to infections. The DH82 cell line was originally reported by Wellman *et al.*¹ as a canine macrophage monocytic cell line that was established from the neoplastic progenitor cells of a canine malignant histiocytosis.

The DH82 cells were cultured in MEM with 10 percent FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were then treated with 100 ng/mL LPS for up to 24 hours and the culture supernatant was collected.

The culture medium sample was assayed according to the *Procarta® Cytokine Assay Kit User Manual for Cell Culture Supernatant Samples*. First, 50 µL of diluted or undiluted sample was added to the capture bead mixture per assay well of the 96-well filter plate and incubated on a shaker at 500 RPM for 60 minutes at room temperature. The plate was washed three times with wash buffer and 25 µL of detection antibody mix was added to each well. After a 30-minute incubation, the plate was washed three times, then 50 µL of SAPE was added to each well. The plate was incubated for another 30 minutes, then washed three times. Next, 120 µL of reading buffer was added to each well and the plate was analyzed using a Luminex® 200 instrument with Bio-Plex Manager™ Software (Bio-Rad, USA).

As expected, there was a significant increase in the proinflammatory cytokines, TNF-α, IL-8, and IL-6 (Figure 2, p. 3). For example, the concentration of IL-6 in the culture medium increased

7.8-fold after four hours of treatment with LPS, and continuously increased 41.7-fold after 24 hours of the treatment. No significant change was detected for untreated samples even after 24 hours. Low levels of IFN-γ were observed in the LPS-untreated samples and IFN-γ concentration in the culture medium did not significantly change with LPS treatment. This supports an established theory that production of IFN-γ is restricted to T lymphocytes (T cells) and natural killer cells (NK cells).

Conclusion

The Procarta® Canine Cytokine Assay Kit is a simple and cost-effective method for measuring cytokines in a multiplex, 96-well-plate format with great specificity, reliability, sensitivity, and precision in less than three hours.

Compared to current methods, the assay enables you to process larger batches of samples in a shorter period, is less labor intensive, and provides a wider range of quantitative data points with a minimal sample volume of 25 µL.

Average recovery values for the serum samples of 111 percent show the assay’s reliability. With CV values below 15 percent for intra- and inter-assay precision, and an absence of significant cross-reactivity, the Procarta Canine Cytokine Assay Kit demonstrates robust and highly specific performance.

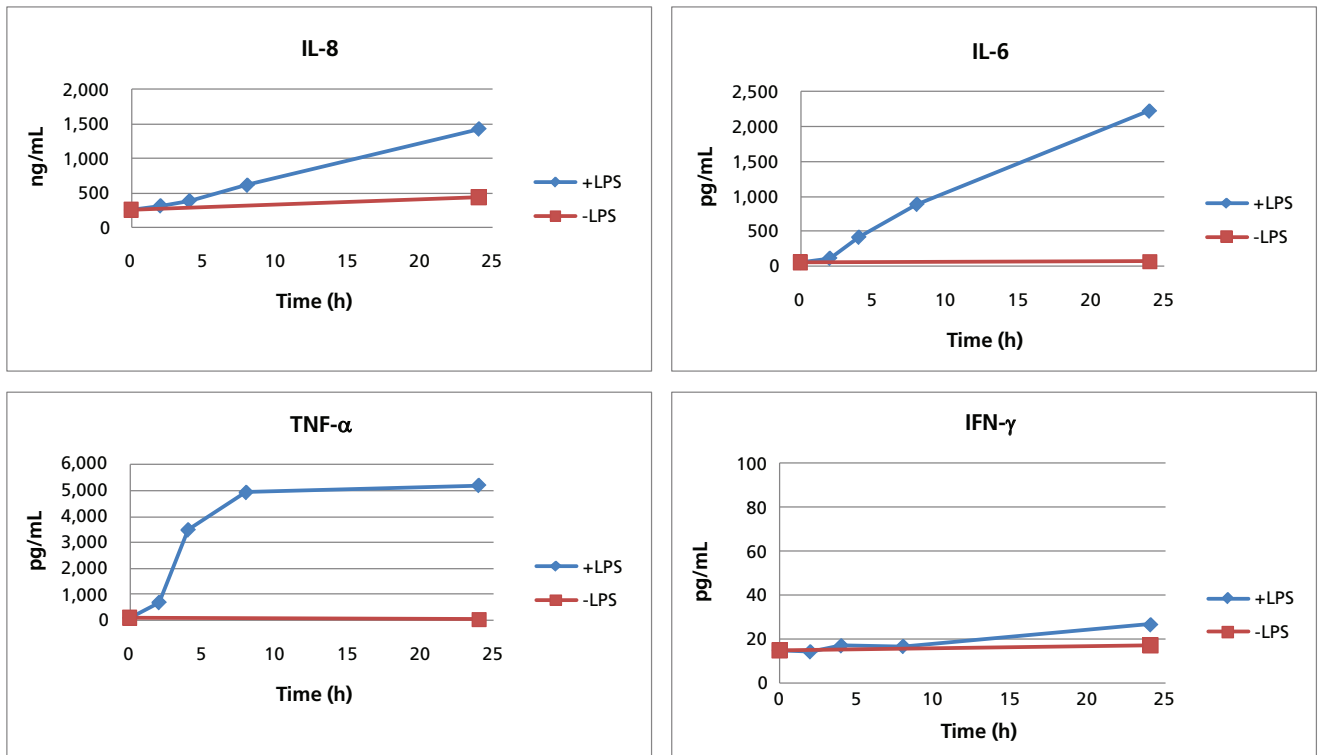
Reference

1. Wellman M. L., Krakowa S., Jacobs R. M., Kociba G. J. A macrophage-monocyte cell line from a dog with malignant histiocytosis. *In Vitro Cellular & Developmental Biology* **24**(3)223-9 (1988).

Table 1: Precision and sensitivity of the assay. Limit of detection (pg/mL), intra- and inter-assay precision (CV), and recovery of the assay (%) were determined for the 12 targets.

	SCF	IL-2	IL-6	IL-8	IL-10	TGFβ1
Limit of detection (pg/mL)	4.1	6.8	7.6	5.1	6.1	1.3
Intra-assay precision (% CV)	3.8	4.1	4.8	3.2	17.9	3.5
Inter-assay precision (% CV)	8.3	11.6	11.8	6.9	19.3	11.4
Spike-in recovery (% average)	108	106	109	102	116	116
Spike-in recovery (% range)	108-126	112-127	101-118	100-113	103-127	99-118
	IL-12p40	NGF	IFN-γ	TNF-α	MCP-1	VEGF
Limit of detection (pg/mL)	6.6	3.3	3.8	0.7	6.5	5.9
Intra-assay precision (% CV)	5.4	3.7	5.6	3.6	5.6	3.79
Inter-assay precision (% CV)	15.6	7.4	9.5	9.2	6.8	7.8
Spike-in recovery (% average)	108	103	122	120	113	108
Spike-in recovery (% range)	95-116	105-122	95-110	112-127	103-115	103-113

Figure 2: Measuring cytokines secreted from canine macrophages into the culture medium. Following the *Procarta® Cytokine Assay User Manual*, 50 μ L of undiluted culture supernatant was assayed for IL-6 and IFN- γ . For TNF- α and IL-8, samples were diluted 100-fold and 50 μ L of the diluted samples was assayed. Blue: treated with LPS; red: untreated.



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