

Procarta[®] Rat Cytokine Assay Kit

Multiplexed quantification of rat 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[®] Rat Cytokine Assay Kit from Affymetrix is a simple, precise, and sensitive method for measuring cytokines in a multiplex format. The assay is based on Luminex[®] xMAP[®] technology and uses fluorescent beads. Affymetrix offers assays using magnetic and non-magnetic beads.

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 30 targets 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.

Figure 1: Typical standard curves for the Procarta Rat Cytokine Assay Kit. Premixed lyophilized antigen standard provided in the kit contains recombinant cytokines in the amounts 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.

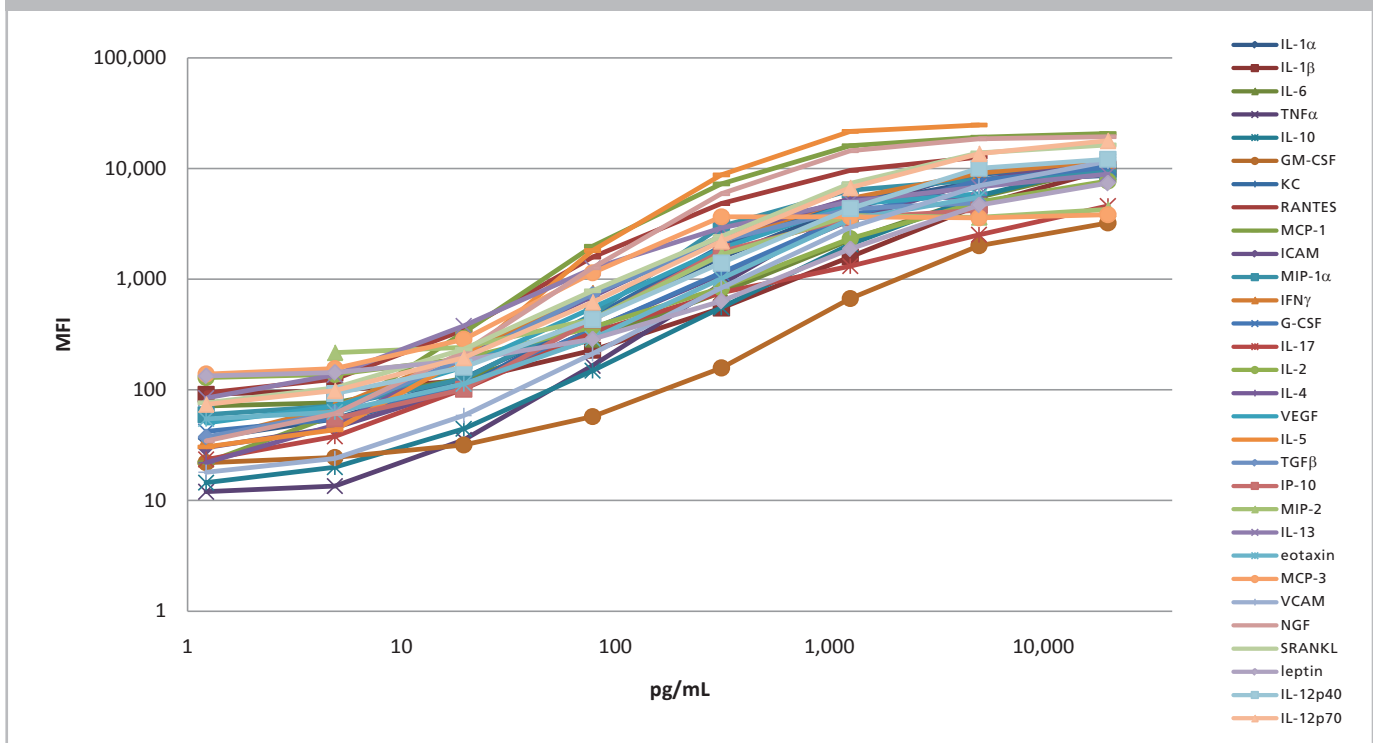


Table 1: Precision and sensitivity of the assay. Limit of detection (pg/mL), intra- and inter-assay precision (% CV), and recovery of the assay (%) were determined for the 12 targets.

	IL-1 α	IL-1 β	IL-6	TNF α	IL-10	GM-CSF	KC	RANTES	MCP-1	ICAM
Limit of detection (pg/mL)	0.1	0.5	6.5	0.7	5.0	2.9	1.4	1.2	1.4	3.9
Intra-assay precision (% CV)	3.9	4.0	3.3	6.2	7.6	3.5	2.0	2.1	4.8	2.8
Inter-assay precision (% CV)	3.3	3.7	2.5	4.0	5.4	4.2	2.8	2.6	4.4	3.4
Spike-in recovery (% average)	104	101	101	103	99	104	96	97	99	100

	MIP-1 α	IFN γ	G-CSF	IL-17	IL-2	IL-4	VEGF	IL-5	TGF β	IP-10
Limit of detection (pg/mL)	5.5	1.4	4.8	3.7	7.5	0.4	0.3	1.9	2.1	0.9
Intra-assay precision (% CV)	1.8	4.4	5.2	9.7	3.2	5.0	5.2	4.0	8.2	4.6
Inter-assay precision (% CV)	2.1	3.2	2.8	7.3	3.2	3.7	4.4	4.1	4.6	2.6
Spike-in recovery (% average)	94	100	99	105	100	97	100	94	97	89

	MIP-2	IL-13	eotaxin	MCP-3	VCAM	NGF	SRANKL	leptin	IL-12p40	IL-12p70
Limit of detection (pg/mL)	5.1	2.5	6.7	1.4	0.6	0.3	0.2	1.8	1.0	5.7
Intra-assay precision (% CV)	6.9	1.6	6.6	7.2	4.3	7.7	3.2	6.2	5.0	4.2
Inter-assay precision (% CV)	3.4	1.9	7.4	3.1	3.7	5.3	3.5	4.0	6.4	7.1
Spike-in recovery (% average)	81	102	103	78	100	104	100	96	98	97

The Procarta[®] Rat Cytokine Assay Kit from Affymetrix enables you to simultaneously detect up to 30 cytokines from rat serum, plasma, cell lysates, or cell culture supernatant samples. Capture antibodies that specifically recognize the target cytokine are conjugated onto magnetic or non-magnetic 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 precision

Figure 1 shows typical standard curves for the Procarta Rat Cytokine Assay Kit (magnetic beads version). The standard curves for 30 targets range from 1.22 to 20,000 pg/mL in the assay well. The limit of detection, shown in Table 1, was between 0.1 and 6.7 pg/mL.

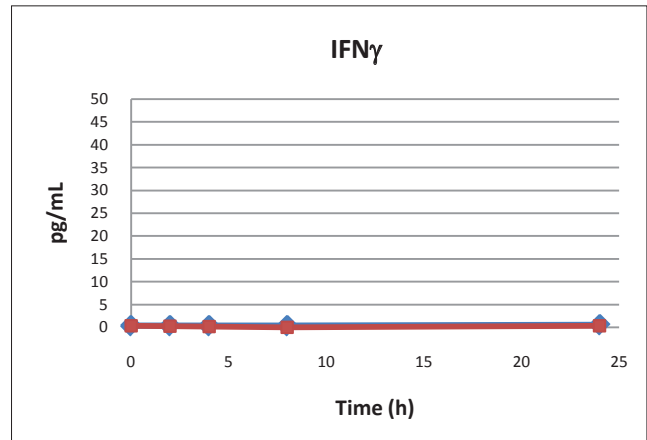
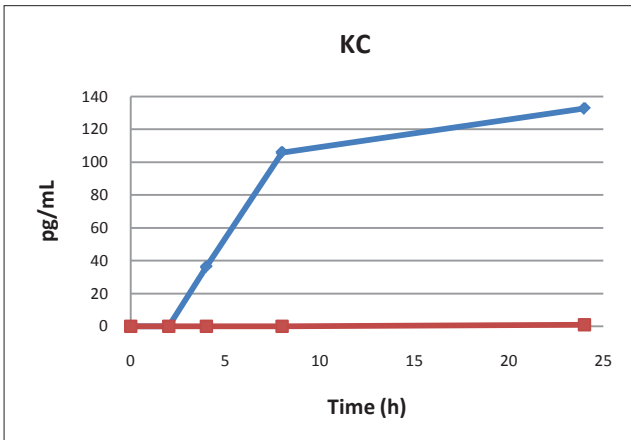
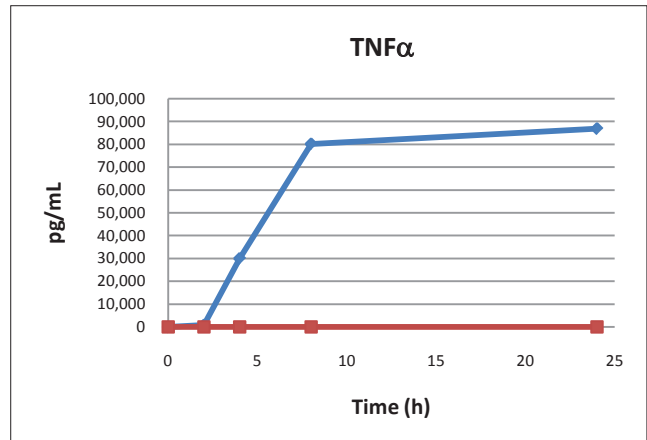
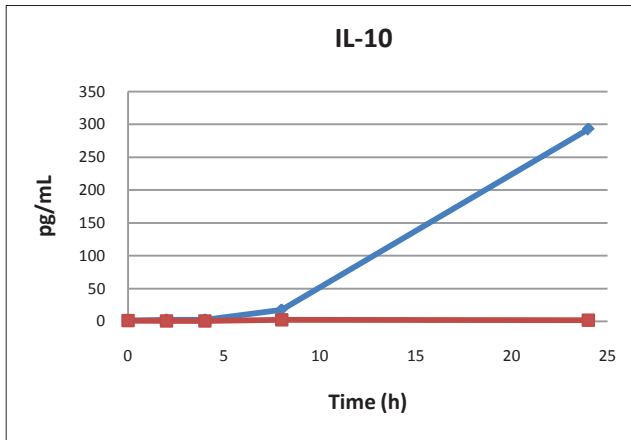
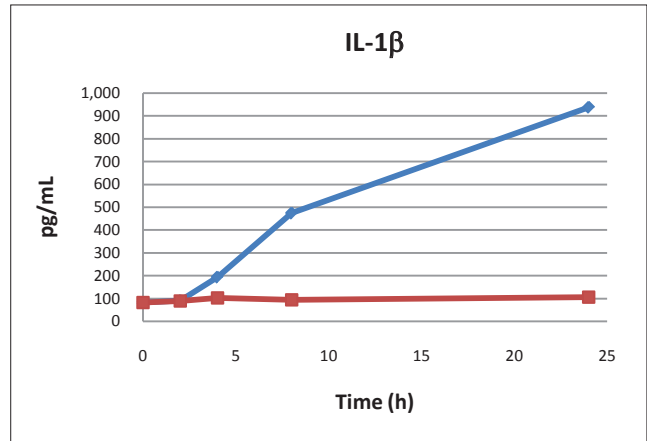
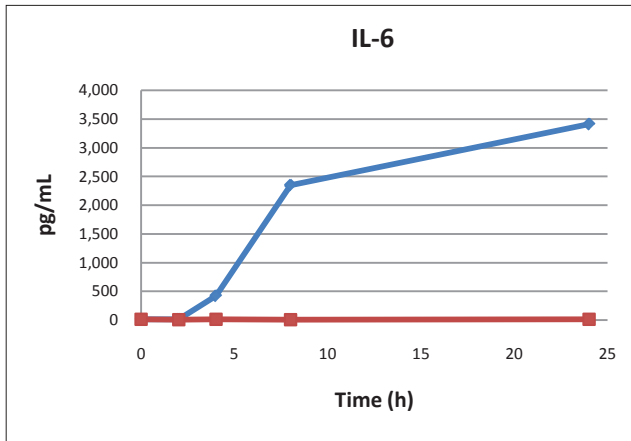
To measure intra- and inter-assay precision, samples of known concentration were tested 20 times on one plate to assess precision within an assay, and five plates were processed (Table 1). All data acquired were processed as a multiplex. The average coefficient of variation (CV) for intra-assay precision was 4.8 percent and for inter-assay precision was 4.0 percent. The average percentage recovery of the concentration of standard antigens, calculated from the 5 PL standard curve fit, was 98 percent (Table 1).

Measuring cytokines secreted from rat macrophages

A rat macrophage cell line, NR8383, treated with lipopolysaccharide (LPS), was used as a model for secretion of pro-inflammatory cytokines under immune response to infections. NR8383 is a continuous line of rat alveolar macrophages that exhibits properties of phagocytosis, nonspecific esterase activity, Fc receptors, oxidative burst, interleukin-1 secretion, and replicative response to exogenous growth factor (Helmke, *et al.*¹). The cells were cultured in advanced RPMI medium containing 10 percent FBS, 100 units/mL penicillin, and 100 μ g/mL streptomycin. Cells were treated with 100 ng/mL LPS for up to 24 hours, and the culture supernatant was collected to analyze secreted cytokines using the Procarta Rat Cytokine Assay Kit.

The culture media samples were assayed according to the Procarta[®] Cytokine Assay Kit User Manual. First, 50 μ L of diluted or undiluted sample was added per assay well to the capture bead mixture of the 96-well filter plate and incubated on a shaker at 500 RPM for 30 minutes at room temperature. The plate was washed three times with wash buffer, and 25 μ L of detection antibody mix was added to each well. After a 30-minute incubation, the plate was washed three times, then 50 μ L of SAPE was added to each well. The plate was incubated for another 30 minutes, washed three times, and 120 μ L of reading buffer was added to each well. The plate was analyzed using a Luminex 200 instrument with Bio-Plex Manager 5.0 Software (Bio-Rad, USA).

Figure 2: Measuring cytokines secreted from rat macrophage into the culture medium. A 50 μ L aliquot of undiluted culture supernatants was assayed for IL-6, IL-1 β , IL-10, KC, and IFN γ following the *Procarta*[®] Cytokine Assay User Manual. For TNF α , samples were diluted 100-fold and 50 μ L of the diluted samples was assayed. Blue: treated with LPS; red: untreated.



As expected, we observed a significant increase in the concentration of pro-inflammatory cytokines, such as TNF α , IL-6, IL-10, and IL-1 β (Figure 2). For example, the concentration of IL-6 in the culture medium increased 36-fold after 4 hours of LPS treatment and continuously increased to 290-fold after 24 hours of treatment, although no significant change was detected for untreated samples even after 24 hours.

However, an extremely low level of IFN γ was observed in the LPS-untreated sample, and the IFN γ concentration in the culture medium did not significantly change with LPS treatment, supporting the established theory that production of IFN γ is restricted to T and NK cells (Figure 2).

Conclusion

The Procarta[®] Rat Cytokine Assay Kit is a simple and cost-effective method for measuring cytokine proteins 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 processing of 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 μ L.

Average recovery values for the serum sample of 98 percent show the assay's reliability. With CV values below 8 percent for intra- and inter-assay precision, and an absence of significant cross-reactivity, the Procarta Rat Cytokine Assay Kit demonstrates robust and highly specific performance.

Reference

1. Helmke R. J., German V. F., Mangos J. A. A continuous alveolar macrophage cell line: comparisons with freshly derived alveolar macrophages. *In Vitro Cellular & Developmental Biology* **25**(1):44-8 (1989).

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