

Procarta® Apolipoprotein Assay Kit

Multiplexed quantification of apolipoproteins

Current methods for the detection and quantitation of apolipoproteins, 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® Apolipoprotein Assay Kit from Affymetrix is a simple, precise, and sensitive method for measuring human apolipoproteins in a multiplexed format. The assay, based on Luminex® xMAP® technology, enables you to detect up to five targets simultaneously.

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 five targets in a single, three-hour reaction

Introduction

Apolipoproteins are proteins that bind to lipids to form lipoproteins, such as low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs), which transport the lipids through the lymphatic and circulatory systems (Figure 1). HDLs, which are considered “good cholesterol,” carry about 30 percent of blood cholesterol in healthy individuals. Higher levels of HDL are associated with decreased cardiovascular problems. LDLs are known as “bad cholesterol” because they can transport lipids into the arteries and thus can increase atherosclerosis in individuals with higher levels of LDL.

Lipids are not soluble in water; however, because of their amphipathic properties, apolipoproteins and other detergent-like molecules such as phospholipids can surround lipids and create water-soluble lipoprotein particles. Thus, they can be carried through water-based lymphatic and circulatory systems. Apolipoproteins also function as enzyme cofactors, receptor ligands, and lipid transfer carriers, regulating the metabolism of lipoproteins and their uptake in tissues.

The Procarta Apolipoprotein Assay Kit from Affymetrix enables you to simultaneously detect up to five apolipoproteins from

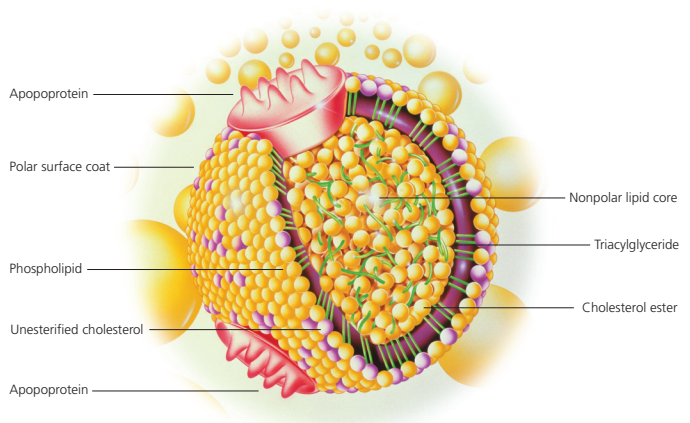


Figure 1: Structure of a lipoprotein. A lipoprotein, such as LDL or HDL, contains both proteins and lipids and transports water-insoluble lipids in the water-based bloodstream. The lipids or their derivatives are covalently or non-covalently bound to the proteins.

human serum and plasma (Table 1). Capture antibodies that specifically recognize target apolipoprotein molecules are conjugated onto fluorescent beads. Each target in the sample binds to a specific capture bead, and the captured target is labeled by a biotinylated anti-apolipoprotein secondary antibody together with streptavidin-PE (SAPE) dye. The fluorescent signal emitted from each biotin-labeled bead is detected using a Luminex-based instrument. The assay is highly sensitive, quantitative, and can be completed in less than three hours.

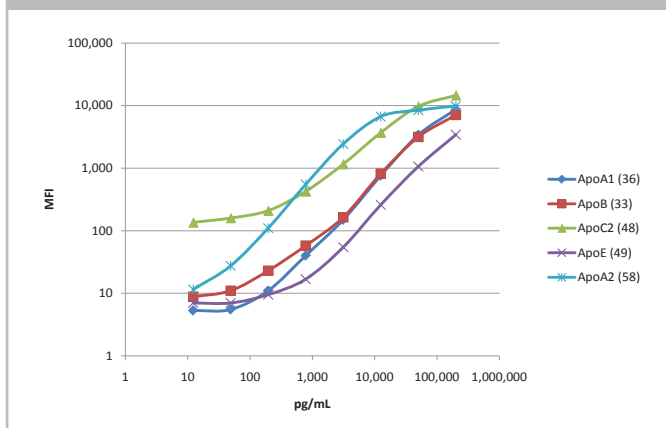
Table 1: Apolipoproteins detected by the assay kit.

Apolipoprotein	Description
ApoA1	Major component of HDL; plays a specific role in lipid metabolism
ApoA2	Second most abundant protein in HDL; defects in this gene may result in apolipoprotein A-II deficiency or hypercholesterolemia
ApoB	Primary component of LDL
ApoC2	A component of LDL that is secreted in plasma and activates the enzyme lipoprotein lipase
ApoE	Essential for normal catabolism of triglyceride-rich lipoprotein constituents

Detection range

Figure 2 shows typical standard curves for the Procarta Apolipoprotein Assay Kit. The standard curves for ApoA1, ApoA2, ApoB, ApoC2, and ApoE range from 12.21 to 200,000 pg/mL in the assay well. These standard curves are suitable for simultaneously detecting all five targets in a single plasma sample. It is not

Figure 2: Typical standard curves for the Procarta Apolipoprotein Assay Kit. Premixed antigen standard provided in the kit contains highly purified apolipoproteins in amounts indicated in the instructions. The premixed standard was reconstituted in assay buffer and four-fold serial dilutions were prepared. Each of the diluted standards was assayed in duplicate.



necessary to prepare different sample dilutions for each target. Plasma samples typically need to be diluted 20,000-fold in assay buffer, and 25 μ L of each diluted sample is used for an assay.

Precision

To measure intra- and inter-assay precision, samples of known concentration were tested 10 times on one plate, and five plates were processed (Table 2). All data acquired were processed as a multiplex.

The coefficient of variation (CV) for intra-assay precision was less than 13 percent, and for inter-assay precision was less than 15 percent. The percentage recovery of the concentration of standard antigens, calculated from the 5 PL standard curve fit, was between 94 and 121 percent.

Detecting apolipoproteins in human plasma samples

Three human plasma samples were diluted 20,000-fold in assay buffer and 25 μ L of diluted samples was assayed according to the *Procarta® Apolipoprotein Assay Kit User Manual*. Next, 25 μ L of diluted samples and 25 μ L of assay buffer per assay well were added 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 and 50 μ L of SAPE was added to each well. The plate was incubated for

another 30 minutes, washed three times, then 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). The results exhibited clear biological differences for each apolipoprotein in the three human plasma samples, as shown in Figure 3.

Conclusion

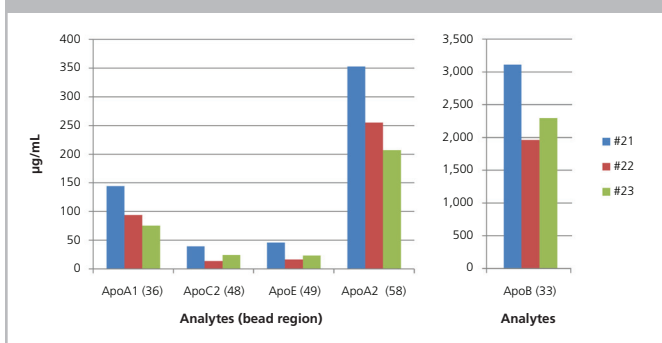
The Procarta® Apolipoprotein Assay Kit is a simple and cost-effective method for measuring apolipoproteins from human serum, plasma, and cell culture supernatant samples in less than three hours. The assay offers a multiplex, 96-well-plate format and shows great specificity, reliability, sensitivity, and precision.

Average recovery values for antigens of 105 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 assay demonstrates highly specific performance.

Table 2: Intra- and inter-assay precision.

	ApoA1	ApoA2	ApoB	ApoC2	ApoE
Intra-assay precision (% CV)	12.45	5.56	6.8	5.29	7.68
Inter-assay precision (% CV)	14.02	5.42	8.37	5.79	7.51
Recovery (% average)	98.2	121.3	94.0	109.5	101.1

Figure 3: Concentration of five apolipoproteins in three human plasma samples. Each sample was assayed in duplicate. Average CV for the sample assays was 7.6 percent.



Ordering information

Part number	Product	Description
PC7007	Procarta® Apolipoprotein Assay Kit	5-plex, 1 plate
PC7008	Procarta® Apolipoprotein Assay Kit	5-plex, 10 plates

Affymetrix, Inc. Tel: +1-888-362-2447 ■ Affymetrix UK Ltd. Tel: +44-(0)1628-552550 ■ Affymetrix Japan K.K. Tel: +81-(0)3-6430-4020
Panomics Products Tel: +1-877-PANOMICS www.panomics.com ■ USB Products Tel: +1-800-321-9322 www.usb.affymetrix.com

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