



Pu·MA System ELISA: Human MCP-1/CCL2

Introduction

Human MCP-1, also known as CCL2, is a member of the CC β chemokine family. It is widely expressed in endothelial cells, smooth muscle cells, and monocytes in response to several atherogenic stimulants such as CD40 ligand, platelet derived growth factor (PDGF), interleukin-1 β (IL-1 β), and oxidized low density lipoprotein. Several recent in vivo studies have disclosed critical roles of MCP-1 in atherosclerosis. In addition, MCP-1 has been implicated in monocytic infiltration of tissues during several inflammatory diseases, and has been implicated in macrophage-mediated tumor growth suppression in mice. Kidney epithelial cells, including glomerular podocytes and tubular cells, make MCP-1 in response to high glucose and advanced glycation end products.

Assay Overview

The workflow for the Pu·MA System cytokine immunoassay is shown in Figure 1. All assay reagents are prepared in advance and then loaded into Pu·MA System flowchips using single or 8-channel pipettes. The wells are conveniently located on standard 384 multiwell plate spacings. The flowchips are now ready to be loaded into the Pu·MA System for “hands-off” processing. The ELISA steps are automatically performed by the system using pre-loaded protocols. Once the assay is complete, absorbance results are read on your Plate Reader.

Pu·MA System Complete Workflow: 2-3 hours “hands-off”



Figure 1. Schematic Pu·MA System assay workflow.

The adaption of existing ELISA kits and/or antibody pairs is straightforward with the Pu·MA System. Assay buffers optimized for the Pu·MA System flowchips are provided for sample and antibody dilutions. Blocking solutions and wash buffers are also provided. The MCP-1 assays are adapted from BioLegend ELISA Max Kits (p/n 438804, 438805, & 438806). The reagents required for the assay are shown in the table.

Assay Procedure

Assay antibody reagents were prepared according to the dilutions shown in the Table using Pu·MA Assay Buffer (PAB). MCP-1 standards were reconstituted according to instructions from BioLegend. A 1:2 serial dilution series of Human MCP-1 Standard was prepared starting at 100 pg/well using PAB. 20 ml of each reagent was added to the appropriate wells (see Fig 3) except for the Stop Solution where 10 ml was dispensed. Four replicates were run per concentration. The flowchips were loaded into a Pu·MA System and processed using the MCP-1 Assay Protocol. Plates were read within 5 minutes of being finished on an absorbance plate reader at 450 nm (Tecan Spectrafluor Plus).

Name	Reagent	Source
Capture Ab	Human MCP-1 ELISA Max Capture Antibody (1:100)	BL
Block	Pu·MA Blocking Buffer	PFI
Sample	Human MCP-1 Standard	BL
1°Ab	Human MCP-1 ELISA Max Detection Antibody (1:1000)	BL
2°Ab	Avidin-HRP (1:2K)	BL
Wash	Pu·MA Wash Buffer	PFI
Substrate	FAST Substrate	PFI
Stop	Pu·MA Stop Solution	PFI

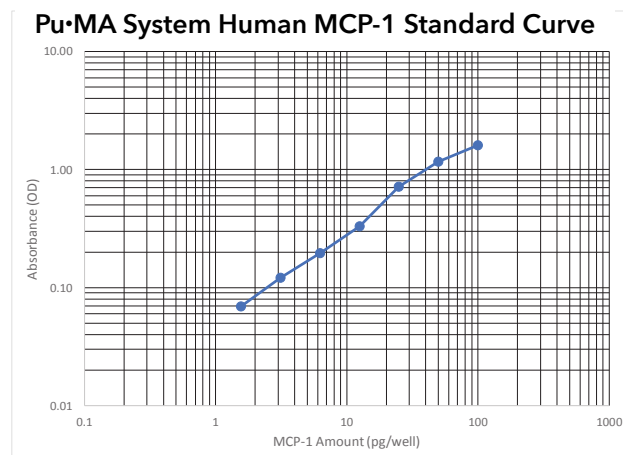
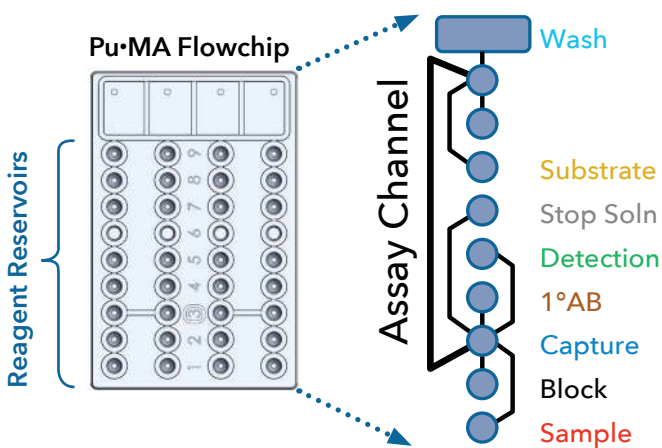


Figure 2. Response of Cytokine Standards for ELISA run on Pu·MA System. ELISA antibody pairs and standards were obtained from BioLegend. Absorbance was read at 450 nm. OD values shown are background subtracted.

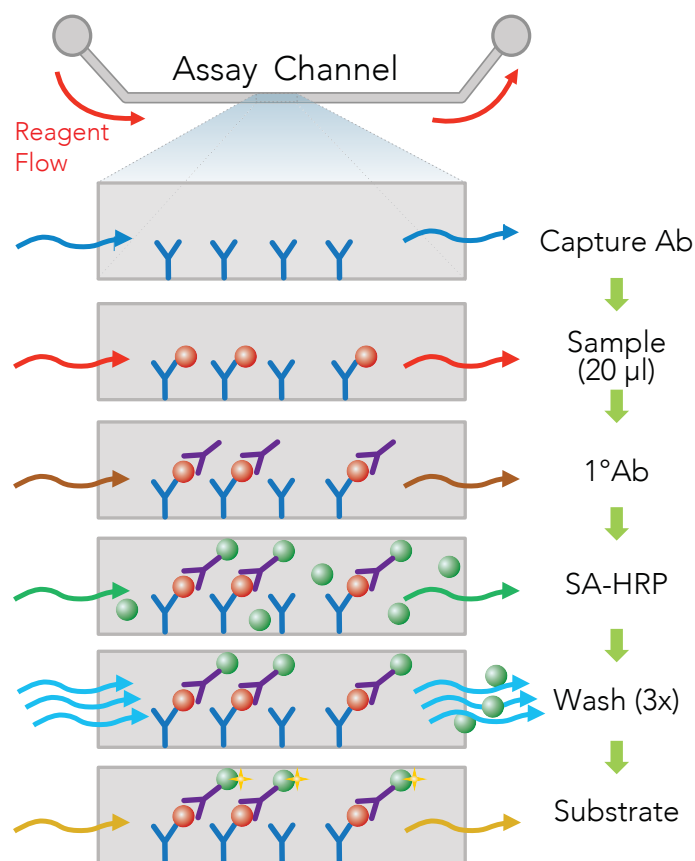
How It Works

The Pu·MA Flowchip and System uses established antibody pairs to perform an automated ELISA. All assay reagents are loaded into reservoirs and then moved one at a time through the "Assay Channel" by the Pu·MA System. Preloaded protocols execute all fluid transfer and incubation steps. The system incorporates patented valveless fluidic switching (VLFS) to precisely control fluid movement in a flowchip. Use of microfluidics reduces both incubation times and reagent volumes.

Reagent Loading Setup



Microfluidic Assay Workflow



- Low sample/reagent usage: < 20 µl/well
- Enhanced kinetics: Faster Assay Results
- Efficient fluid removal: Less Wash Required

Figure 3. Pu·MA Flowchip reagent loading setup.

Pu·MA System



- Compact benchtop system
- Easy top-loading of flowchips
- 1 to 3 hr Processing Time

Pu·MA Software



- Touchscreen-driven interface
- Preloaded assay protocols
- Simple Select and Run operation

Reagents & Flowchips



- Active Coat Flowchips with holder
- Optimized buffers and reagents
- Store at 4°C