PROTEIN FLUIDICS

PRODUCT NOTE 3

Pu·MA System ELISA: Human Interleukin-8

Introduction

Human Interleukin-8 (IL-8), also known as CXCL8, is a chemokine that is upregulated at sites of inflammation where it promotes neutrophil infiltration and activation. It is a member of the alpha (C-X-C) subfamily of chemokines. In response to proinflammatory stimuli, IL-8 is produced by monocytes, macrophages, T cells, neutrophils, and fibroblasts. IL-8 can form homodimers and heterodimers with CXCL4/PF4. Its bioactivity is regulated by proteolytic truncations, citrullination, and the decoy receptor DARC. IL-8 signals through CXCR1/IL-8 RA, which is also used by CXCL6, and through CXCR2/IL-8 RB, which is used by multiple CXC chemokines.

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 IL-8 assays are adapted from BioLegend ELISA Max Kits (p/n 431501, 431502, & 431503). 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). IL-8 standards were reconstituted according to instruction provided by BioLegend. A 1:2 serial dilution series of Human IL-8 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 IL-8 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 IL-8 ELISA Max Capture Antibody (1:100)	BL
Block	Pu·MA Blocking Buffer	PFI
Sample	Human IL-8 Standard	BL
1°Ab	Human IL-8 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

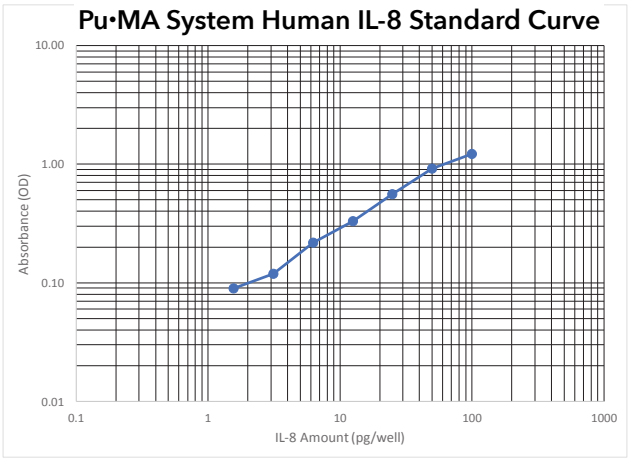
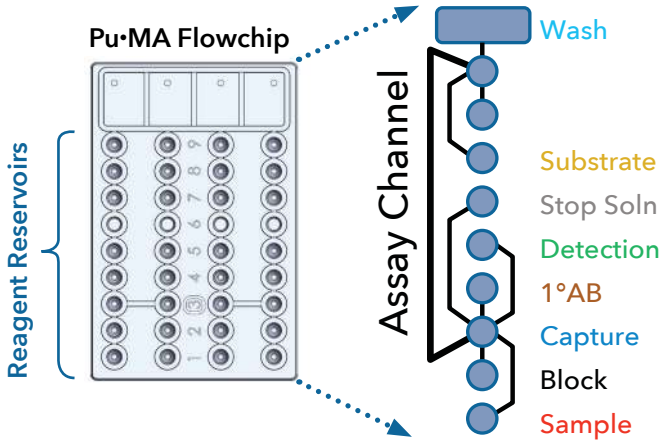


Figure 2. Response of Cytokine Standards for ELISA run on Pu·MA System. ELISA antibody pairs and standards were obtained from BioLegend. Absorbance was read at 450nm. OD values shown are background subtracted.

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 valve-less fluidic switching (VLFS) to precisely control fluid movement in a flowchip. Use of microfluidics reduces both incubation times and reagent volumes.

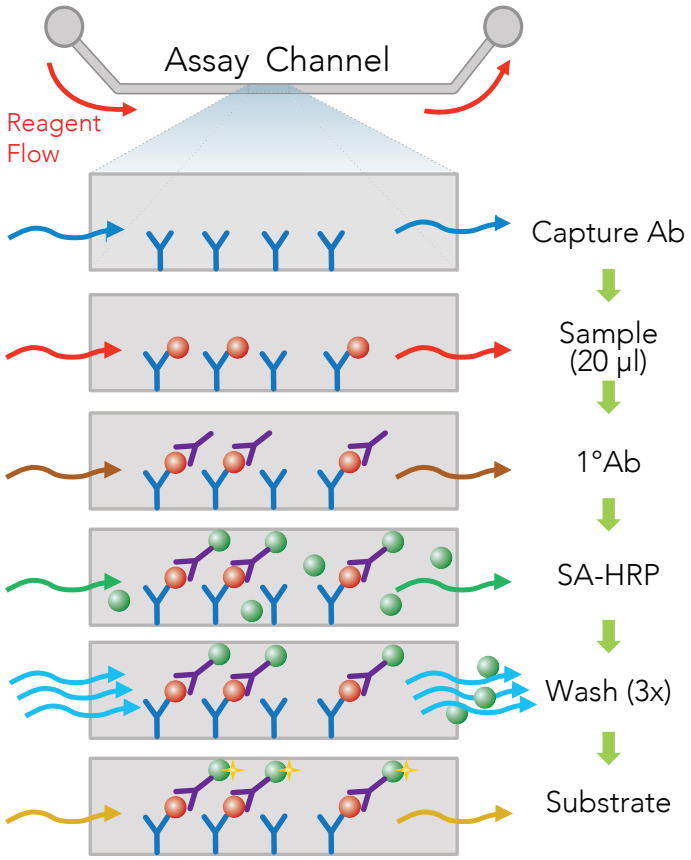
Reagent Loading Setup



- 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.

Microfluidic Assay Workflow



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

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875 Cowan Road, Suite B, Burlingame, CA 94010 U.S.A.
+1-650-529-5080 • www.proteinfluidics.com

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