PROTEIN FLUIDICS

PRODUCT NOTE 4

Pu·MA System ELISA: Human TNFα

Introduction

Human TNF-α (Tumor Necrosis Factor-Alpha), also termed Cachectin, Cytotoxic Factor (CF), CTX, Hemorrhagic Factor, Macrophage-Derived Cytotoxic Factor, and Macrophage Cytotoxic Factor (MCF), is a potent multifunctional cytokine which can exert regulatory, inflammatory and cytotoxic effects on a wide range of normal lymphoid and non-lymphoid cells and tumor cells. TNF-α is produced by a wide variety of immune and epithelial cell types. TNF-α regulates lymphoid tissue development through control of apoptosis. It also promotes inflammatory responses by inducing the activation of vascular endothelial cells and macrophages. TNF-α is a key cytokine in the development of several inflammatory disorders. It contributes to the development of type 2 diabetes through its effects on insulin resistance and fatty acid metabolism.

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 TNF-α assays are adapted from BioLegend ELISA Max Kits (p/n 430201, 4330202, & 430203). 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). TNF-α standards were reconstituted according to instructions from BioLegend. A 1:2 serial dilution series of Human TNF-α 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 TNF-α 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 TNF-α ELISA Max Capture Antibody (1:25)	BL
Block	Pu·MA Blocking Buffer	PFI
Sample	Human TNF-α Standard	BL
1°Ab	Human TNF-α ELISA Max Detection Antibody (1:250)	BL
2°Ab	Avidin-HRP (1:5K)	BL
Wash	Pu·MA Wash Buffer	PFI
Substrate	FAST Substrate	PFI
Stop	Pu·MA Stop Solution	PFI

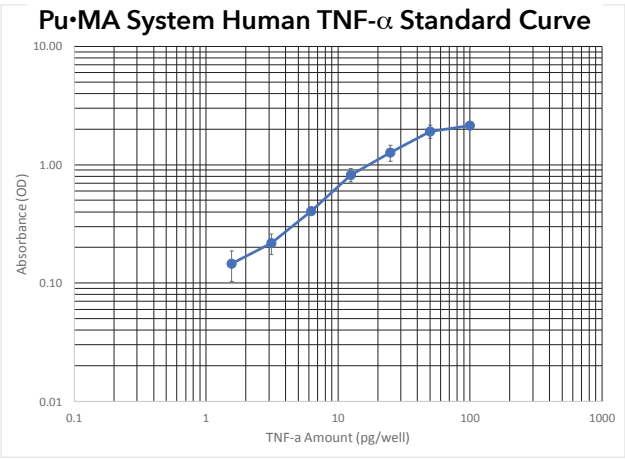
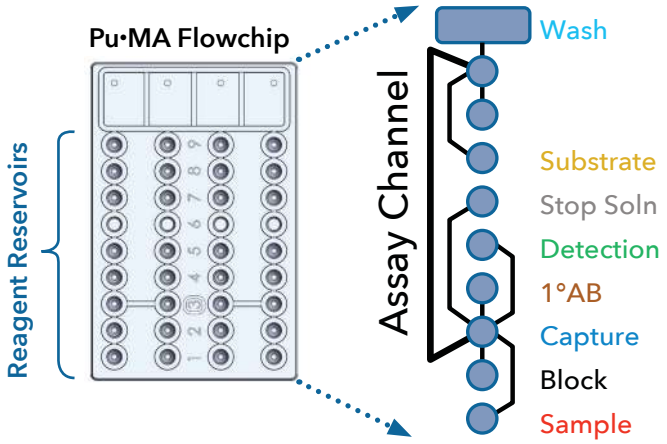


Figure 2. Response of Cytokine Standards for ELISA run on Pu·MA System. ELISA antibody pairs and standards were obtained from BioLegend. Absorbance was read at 450nm. OD values shown are background subtracted.

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.

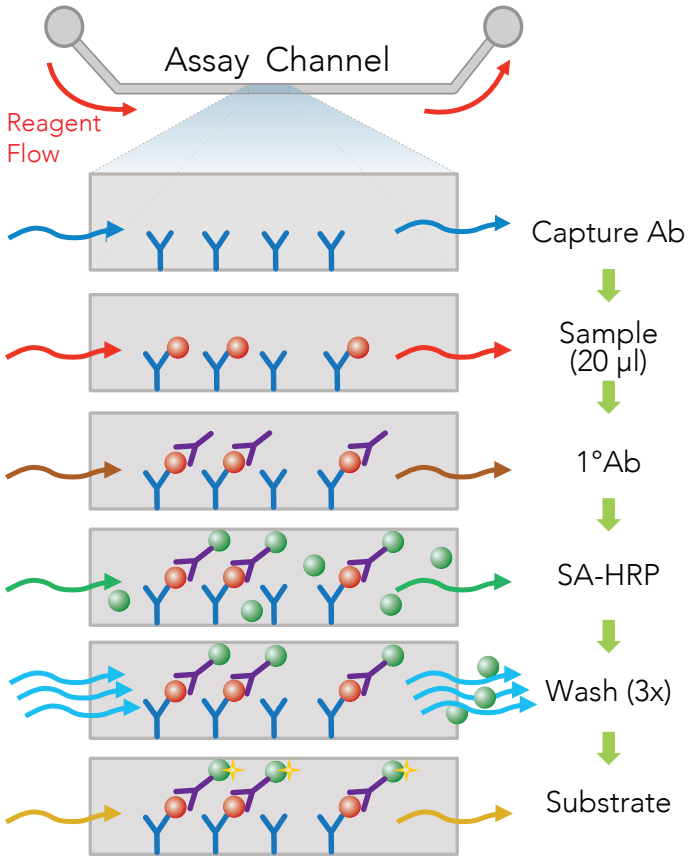
Reagent Loading Setup



- 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

PROTEIN FLUIDICS

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

Rev 092018-1