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Multiplexing Protein and Gene Level Measurements on a Single Luminex Platform

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ABSTRACT

Background: The ability to accurately measure both proteins and genes is expected for comprehensive analysis from a single sample. Bottlenecks to multiplexing assays using a single starting sample include limited sample volume, time consuming experimental procedures, and complicated data analysis. Here, we utilize Luminex® xMAP® technology to measure multiple proteins or genes in a single well. Purpose: Our study examines ProcartaPlex and QuantiGene Plex assays to provide both protein and gene expression data from the same starting sample. Experimental procedures: We demonstrate two high throughput assays measuring genes and proteins and run on a Luminex platform. Human peripheral blood mononuclear cells (hPBMCs) were treated with lipopolysaccharide (LPS) and harvested at 24 and 72 hours. The treated cells were centrifuged and secreted cytokines were measured using the ProcartaPlex Human 65-plex Cytokine Panel, and the cell pellets were lysed and intracellular mRNA was measured with the QuantiGene Plex Human Cytokine Panel. Summary of data: Upon further examination, a subset of gene expression and analyte levels corresponds, namely IL-1β, IL-6, TNF-α, and MIP-1b. Conclusion: These results show that sample can be conserved and produce targeted results.

INTRODUCTION

Screening assays are both time consuming to set-up and execute, and there is a good amount of variability between users (Lamerdin, 2016). Using xMAP technology to measure protein and gene levels on a Luminex instrument, this overcomes previous limitations as these assays are sensitive, specific to a target, quality control tested and easy-to-learn and perform for the end user. ProcartaPlex and QuantiGene assays respectively measure protein and gene expression and provide high-level multiplexing to improve the discovery workflow and screening process (Dunbar, 2005). These assays consumes very little sample, while maximizing the output of data at the proteomic and genomic level.

Table 1. High-throughput protein and gene expression assays

	Invitrogen™ ProcartaPlex™ assay	Invitrogen™ QuantiGene™ Plex assay
Intra-assay CV	≤15%	≤15%
Inter-assay CV	≤15%	≤15%
Linearity	3–5 logs	
Multiplex level	Up to 80	
Formats	96- and 384-well	
Sample types	Serum, plasma, CCS, CSF	RNA, cell/blood/FFPE lysates, tissue homogenates
Species	Human, mouse, rat, canine, porcine, nonhuman primate	Any
Compatible Luminex® instruments	MAGPIX®, Luminex® 200™, FLEXMAP 3D®, xMAP INTELLIFLEX™	
Sample volume	6.3–50 µL	20–80 µL

Invitrogen QuantiGene assay vs. traditional qPCR workflow

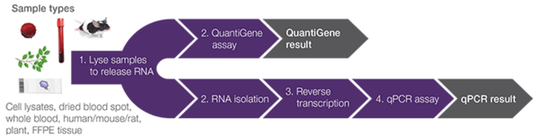
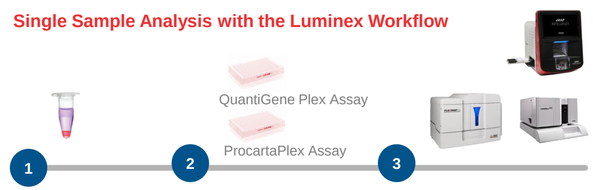


Figure 1: QuantiGene workflow is shorter than traditional qPCR. Traditional qPCR workflow requires isolation and amplification of targeted sequences. QuantiGene assay workflow does not have an amplification step for the gene itself. Instead, branched DNA (bDNA) amplifies the signal.

MATERIALS AND METHODS

Single Sample Analysis with the Luminex Workflow



Human PBMCs were then stimulated with 10 µg/mL LPS at 24 (d1), 48, and 72 hours (d3).

Cells were lysed using Invitrogen™ QuantiGene™ Sample Processing Kit for cultured cells (Thermo Fisher Scientific Cat. No. QS0100) at d1 and d3, and supernatants (stored frozen at -80° C) were then profiled

Plates were read on Luminex instruments including The Luminex LX200 and FLEXMAP 3D instrument with xPONENT® software

Figure 2: Protocol overview for ProcartaPlex and QuantiGene Assays. Human PBMCs were isolated and lysed after treatment. That sample was thereafter used for both protein and gene expression analysis using QuantiGene and ProcartaPlex Assays. Samples were read using Luminex instruments and analyzed with xPONENT software.

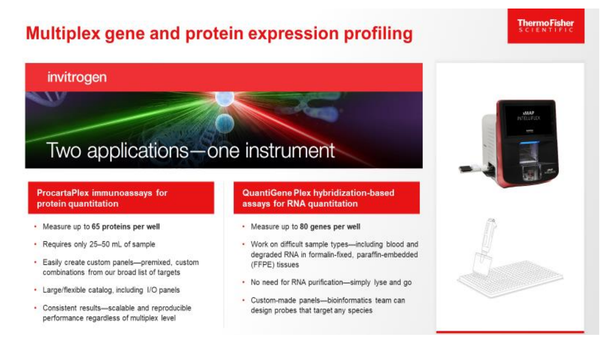
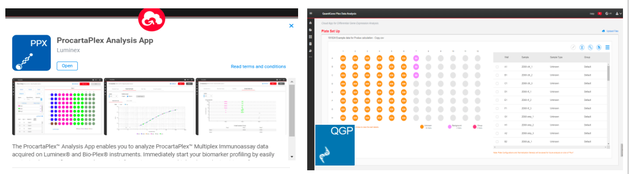


Figure 3: Gene and protein expression analysis on the Luminex platform. Comparison and key benefits of ProcartaPlex and QuantiGene Plex assays.

Data analysis



thermofisher.com/ppxanalysisapp thermofisher.com/QGanalysisapp

Figure 4: Data can be conveniently analyzed using Thermo Fisher Scientific's Connect platform that includes data analysis apps for ProcartaPlex and QuantiGene Plex assay data, offering a complete workflow for in depth data analysis and visualization.

RESULTS

Protein Level Data with ProcartaPlex 65-Plex

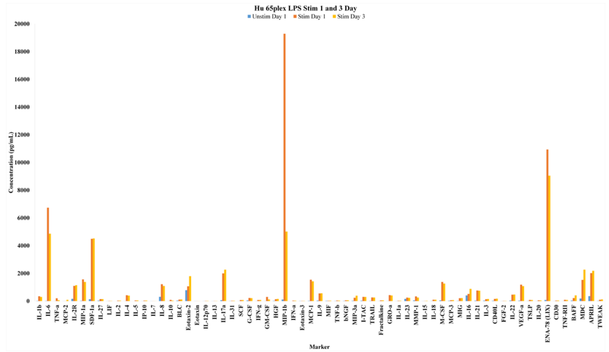


Figure 5: 21 targets showed 10-fold or greater after a 3-day LPS-stimulation. The ProcartaPlex 65-plex data was analyzed using the xPonent software. A 5PL (weighted) curve fit was used for the generation of the 7-pt standard curve. The data was analyzed for precision, accuracy and bead count.

Gene Expression Data with Custom QuantiGene Assay

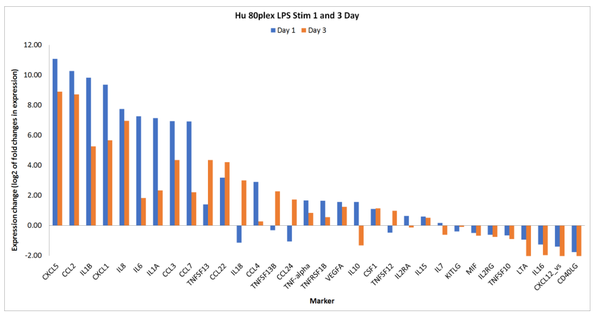


Figure 6: 32 gene targets showed expression changes with LPS-stimulation. Raw MFI data from the QuantiGene Plex assay run were normalized to the geometric mean of the 6 most stable reference genes according to geNorm (out of 12 reference genes in the panel).

Protein and Gene Expression Correlation on the Luminex Platform

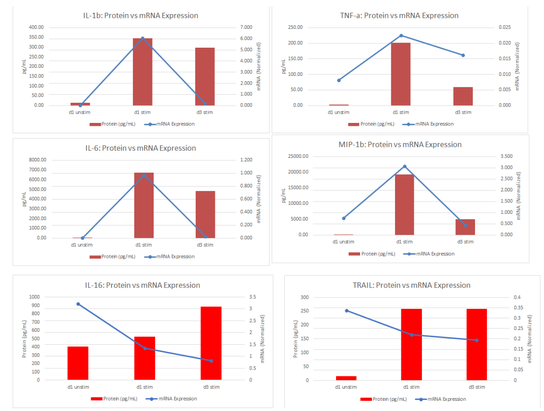


Figure 7: Correlation of protein and mRNA expression at day 1 and 3 post LPS-stimulation.(A) Protein and gene expression levels with correlating trends. (B) Diverging protein and gene expression levels.

CONCLUSIONS

Complementing ProcartaPlex assay with QuantiGene mRNA assay can provide a more holistic view of the investigation research study. Often times, mRNA expression and protein expression alone may not tell a complete story as the mRNA levels may not translate to protein. Adding QuantiGene Plex assays to the ProcartaPlex automated workflow is an amenable solution to meet the high-throughput screening needs for discovery research.

REFERENCES

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