

Multiplex Measurement of Biomarkers in Human Breast Cancer Cell Culture Supernates

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ABSTRACT

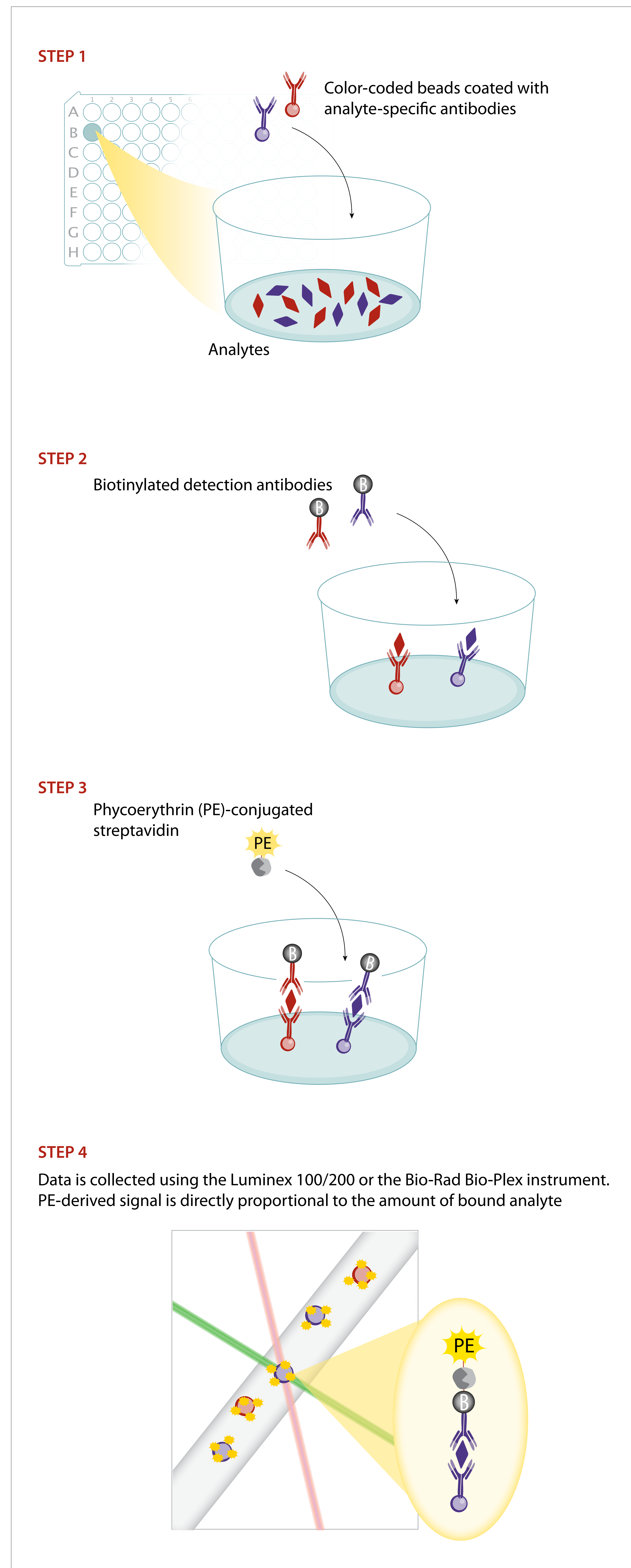
The mixture of biomarkers that is produced in the tumor micro-environment has an important role in cancer pathogenesis. For example, cytokines that are released in response to infection, inflammation, or immunity can function to either promote or inhibit tumor development and progression. The multiplex immunoassay has become an efficient tool both for rapid screening and more in-depth analysis of cancer cell supernates, while requiring only a single, small sample volume. In this study, the VersaMAP™ Development System was used to rapidly and simultaneously screen for human biomarkers in supernates from six human breast cancer cell lines. Fifteen positive biomarkers were identified and biomarker profiles were established for each of the cell lines.

The VersaMAP Development System, developed by R&D Systems, Inc., is a customized assay system designed for the simultaneous measurement of multiple biomarkers from a single 50 µL sample. It offers the ability to measure up to 38 biomarkers chosen by the user from a continually expanding library of analytes. The assay is available in a custom pre-mixed form, giving the user total flexibility to define their multiplex panel. The assay system is a bead-based multiplex immunoassay designed for use with a Luminex® or Bio-Rad® Bio-Plex® analyzer.

INTRODUCTION

Cytokines are pleiotropic extracellular signaling molecules that influence myriad physiological and pathological processes. Released from a variety of cells, their actions affect cellular growth, differentiation, gene expression, migration, immunity, and inflammation. In most biological processes, multiple cytokines operate in a large network where the action of one cytokine is regulated by the presence or absence of other cytokines. Studying the underlying signaling cascades is further complicated by the fact that many cytokines modulate the production of other cytokines. The VersaMAP Development System is a multiplex bead-based assay designed for use with a Luminex or Bio-Rad Bio-Plex analyzer that has recently been expanded to simultaneously detect up to 38 analytes. The user selects any combination of analytes from the R&D Systems VersaMAP listing. VersaMAP Development Systems include 2 x 96-well microplates, analyte-specific, antibody-coated beads, and biotinylated detection antibodies. To increase usability and decrease experimental variability, beads and biotinylated detection antibodies are premixed in respective vials by R&D Systems.

ASSAY PROCEDURE



METHODS

The MDA-MB-435, MCF-7, T47D, ZR-75-1, SK-BR-3 and MDA-MB-231 human breast cancer cell lines were grown to 100% confluence in T-175 flasks. The culture supernates were clarified by centrifugation and analyzed using the VersaMAP Development System (Catalog # VMAPH, R&D Systems, Inc.) for the following biomarkers in a single 38-plex assay.

ANALYTE	STANDARD CURVE RANGE*	ANALYTE	STANDARD CURVE RANGE*	ANALYTE	STANDARD CURVE RANGE*	ANALYTE	STANDARD CURVE RANGE*
Adiponectin	618 - 150275 pg/mL	IL-1α	9 - 2068 pg/mL	IL-17	19 - 4616 pg/mL	MMP-8	391 - 95020 pg/mL
Complement Factor D	747 - 181433 pg/mL	IL-1β	8 - 1987 pg/mL	I-TAC	120 - 29145 pg/mL	MMP-13	301 - 73156 pg/mL
C-Reactive Protein/CRP	129 - 31401 pg/mL	IL-1ra	19 - 4642 pg/mL	Leptin	447 - 108558 pg/mL	P-Selectin	219 - 53248 pg/mL
E-Selectin	330 - 80077 pg/mL	IL-2	17 - 4250 pg/mL	MCP-1	13 - 3142 pg/mL	RANTES	8 - 1854 pg/mL
ENA-78	45 - 10996 pg/mL	IL-4	22 - 5317 pg/mL	MIP-1α	69 - 16720 pg/mL	TNF-α	18 - 4470 pg/mL
FGF basic	23 - 5605 pg/mL	IL-5	8 - 1903 pg/mL	MIP-1β	29 - 6987 pg/mL	Thrombopoietin/Tpo	109 - 26601 pg/mL
G-CSF	35 - 8527 pg/mL	IL-6	18 - 4474 pg/mL	MMP-1	24 - 5821 pg/mL	VCAM-1	5694 - 1383583 pg/mL
GM-CSF	72 - 17472 pg/mL	IL-8	14 - 3290 pg/mL	MMP-2	227 - 55101 pg/mL	VEGF	16 - 3995 pg/mL
ICAM-1	1323 - 321571 pg/mL	IL-10	9 - 2223 pg/mL	MMP-3	47 - 11337 pg/mL		
IFN-γ	27 - 6620 pg/mL	IL-12 p70	258 - 62606 pg/mL	MMP-7	441 - 107217 pg/mL		

*A standard curve must be generated each time an assay is run, utilizing values from the Certificate of Analysis provided with each VersaMAP Development System.

RESULTS

Figure 1.

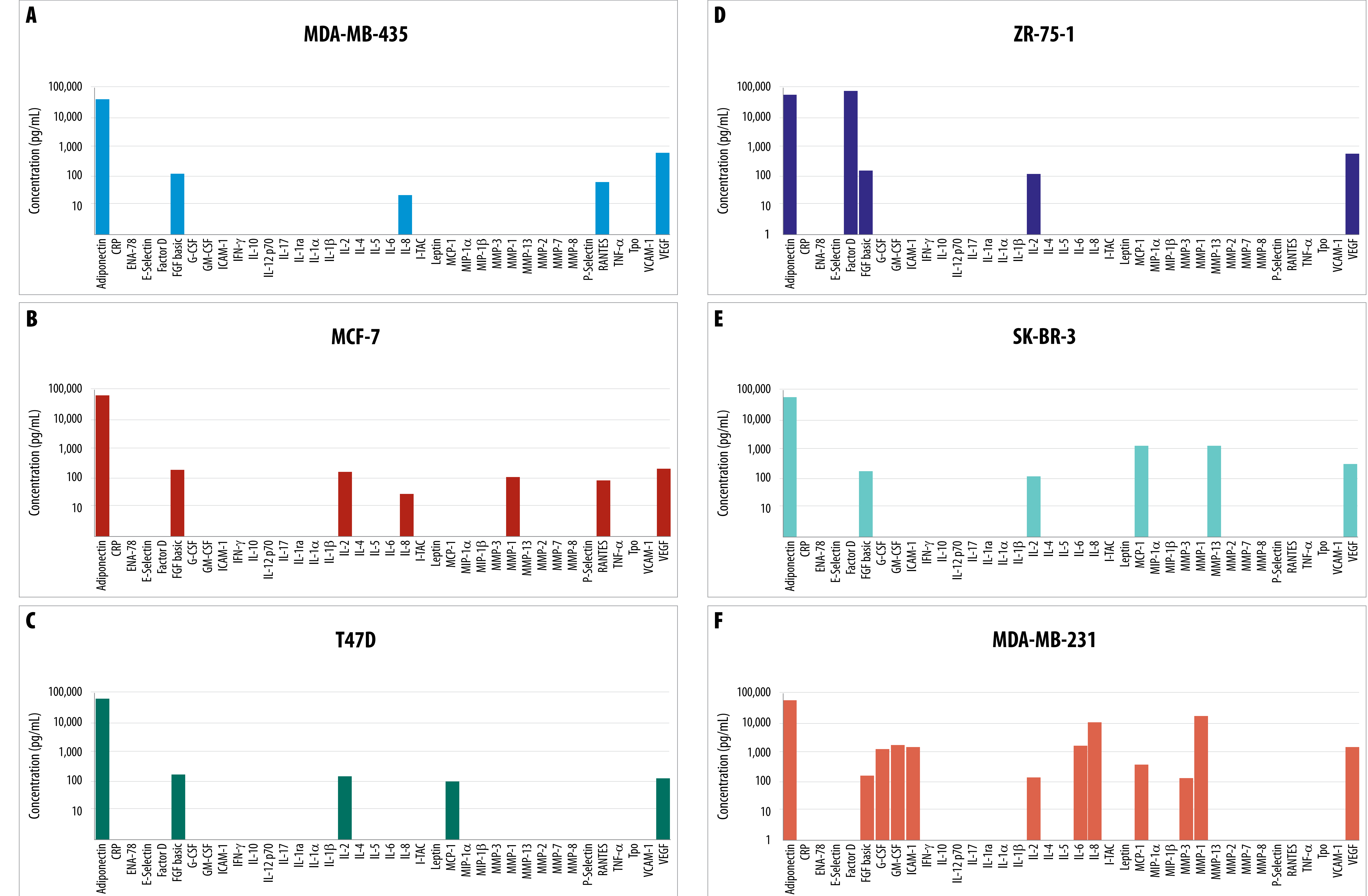


Figure 1. Biomarker profiles of six breast cancer cell supernates assayed as a single multiplex using the VersaMAP Development System. Supernates from the MDA-MB 435 (A), MCF-7 (B), T47D (C), ZR-75-1 (D), SK-BR-3 (E), and MDA-MB 231 (F) human breast cancer cell lines were analyzed for biomarker expression (Table 1) in 96-well microplates using the VersaMAP Development System. The graphs show biomarker concentrations for each cell line analyzed (A-F).

CONCLUSION

Measurement of 38 cytokines in each of these 6 human breast cancer cell line samples using the VersaMAP Development System was accomplished in a single assay using only 50 µL of each sample. Thus, VersaMAP offers an efficient method to assess cytokine profiles with less operator time, less sample consumption, and lower cost per result compared to conventional ELISAs.