Application Note

SOMAmer® Reagents for Analysis of Oncology Biomarkers by Flow Cytometry

Introduction

SOMAmer® reagents (Slow Off-rate Modified Aptamers) are DNA-based high affinity (average KD < 1 nM) protein binding reagents with proprietary chemical modifications that provide hydrophobic characteristics not present in natural DNA. These modifications enhance protein binding through direct hydrophobic contacts with the target protein, resulting in increased binding affinities and slower complex dissociation rates compared to unmodified aptamers.

Due to their high specificity and strong affinity for protein targets, SOMAmer reagents can be used in many life science research applications that traditionally rely on antibodies. Since SOMAmer reagents are chemically-synthesized modified DNA, product consistency and stability are superior to antibodies. Antibodies are produced in fundamentally variable biological systems and suffer from many of the stability issues inherent to protein-based reagents.

Flow cytometry is used as a tool to measure and separate cells based on size, morphology and protein expression level. Protein expression level is traditionally detected either by target-specific primary antibodies conjugated to a fluorescent reporter molecule or with a primary antibody and fluorescent secondary antibody combination. We have used SOMAmer reagents with a 5' biotin functional group in conjunction with fluorescently-labeled streptavidin as the labeling strategy. This technical note describes the use of SOMAmer reagents for measuring, by flow cytometry, the cell surface expression levels of three receptors implicated in cancer biology: Epidermal growth factor receptor (EGFR or ERBB1), Receptor tyrosine kinase erbB2 (ERBB2), and Programmed cell death 1 ligand 1 (PD-L1 or B7-H1).

Materials and Methods

Cell culture

Cell lines SKBR-3, MDA-MB-231, MCF-7, and MDA-MB-468 were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in DMEM supplemented with 10% Fetal Bovine Serum and 10mM L-Glutamine according to recommended protocols.

SOMAmer Reagents

SOMAmer reagents against ERBB2 (product #910-00029), ERBB1 (product #910-00037), B7-H1 (product #910-00235) and Coagulation Factor VII (product #910-00116) were heated to 95° C for 3 minutes, then slowly cooled in a 25° C water bath for 20 minutes prior to dilution in cell staining buffer. SOMAmer reagents were diluted 1 µM in 5 mM HEPES, 1 mM EDTA, pH 8 for heat and cool procedure.

Polyanionic Competitor

Inclusion of a competitor during or after cell labeling can be valuable in distinguishing between specific and non-specific binding in all SOMAmer reagent applications. While many applications do not involve high levels of proteins with high random DNA affinity, they often include very high concentrations of proteins with modest affinity (e.g., some highly abundant plasma proteins that can bind DNA). Addition of a competitor can help reduce artifacts that may arise from random DNA binding layered on specific SOMAmer® reagent binding. SomaLogic's Polyanionic Competitor [Product Number 910-00001] or dextran sulfate at concentrations ranging from 0.1 µM to 10 mM should be evaluated for use in specific applications (see SomaLogic Application Note on Polyanionic Competition). In these experiments, 10 µM SomaLogic Polyanionic Competitor and 2 mM Dextran Sulfate were used during incubation of cells with SOMAmer reagents.

Cell labeling

Culture medium was aspirated from cell monolayers and cells were washed one time with PBS. 2x106 cells were detached using PBS-based Enzyme-free Cell Dissociation Buffer (Thermo Fisher, catalog #13151-014). Cells were harvested, pelleted by centrifugation at 300 x g for 2 minutes and washed once with ice cold Wash Buffer (PBS with 5 mM MgCl2 and 1% BSA). Cells were resuspended in 100 µL of SOMAmer labeling buffer (PBS with 5 mM MgCl2, 1% BSA, 10 µM SomaLogic Polyanionic Competitor, and 2 mM Dextran Sulfate) containing 100 nM of SOMAmer® Reagent and incubated for 45 minutes at 4° C with gentle rocking. Cells were then washed 3 times with 100 µL of Wash Buffer, pelleting by centrifugation at 300 x g at 4° C between each wash. After the final wash, cells were resuspended in 100 µL of Wash buffer containing 1.25 ng/µL of Streptavidin-AlexaFluor 647 conjugate and incubated for 30 minutes at 4° C with gentle rocking. Cells were then washed 3 times with 100 µL of Wash Buffer, pelleting by centrifugation at 300 x g at 4° C between each wash. Labeled and washed cells were resuspended in 100 µL of Wash Buffer with 1% Formaldehyde prior to data acquisition.

Flow Cytometry Data Acquisition

Data were acquired on a BD Accuri C6 flow cytometer by gating conservatively on the live cell population on the basis of forward and side light scatter. The mean fluorescence intensity (MFI) in the FL-4 channel, measuring AlexaFluor 647 fluorescence, was calculated for 10,000 events in the live cell gate.

Flow Cytometry Data Analysis

All data were analyzed using BD CSampler™ Software.

Results

For all experiments, we used a no SOMAmer reagent control to determine the fluorescence contribution of the Streptavidin-AlexaFluor 647 conjugate. We also used a Coagulation Factor VII SOMAmer reagent which targets a soluble factor that should not be present on the cell lines being tested. This irrelevant SOMAmer reagent control was used to determine the amount of fluorescence that could be attributed to non-specific SOMAmer reagent binding to cells. In all cases, the fluorescence intensity of the no SOMAmer reagent and irrelevant SOMAmer reagent controls were nearly superimposable (Figures 1-3, panel A, red and black histogram traces). This result indicates a non-detectable amount of non-specific SOMAmer reagent-cell surface interactions. It is important to note the use of polyanionic competitor during incubation of cells with SOMAmer reagents in order to discourage any such non-specific interactions. We conclude that any fluorescence measured above these control levels should be attributed to specific binding of the SOMAmer reagent to its target cell surface protein.

In the case of the ERBB1-specific SOMAmer reagent, we observed a 12-fold increase in mean fluorescence intensity (MFI) when the ERBB1 SOMAmer reagent was used to label MDA-MB-468 cells, which express high levels of ERBB1 versus a known ERBB1 low cell line, MCF-7 1 (Figure 1). The ERBB2-specific SOMAmer reagent labeled the ERBB2 high cell line, SKBR-3, 240-fold more intensely than the ERBB2 low cell line, MDA-MB-231 2 (Figure 2). Finally, the SOMAmer reagent specific for B7-H1 (also known as PD-L1) labeled B7-H1-expressing MDA-MB-231 cells 12-fold more intensely than the B7-H1 low expressing cell line, MCF-7 3(Figure 3).

Taken together, these results indicate that the ERBB1, ERBB2, and B7-H1 SOMAmer reagents can be used to efficiently and specifically label cell surface receptors for quantitative fluorescence analysis by flow cytometry.

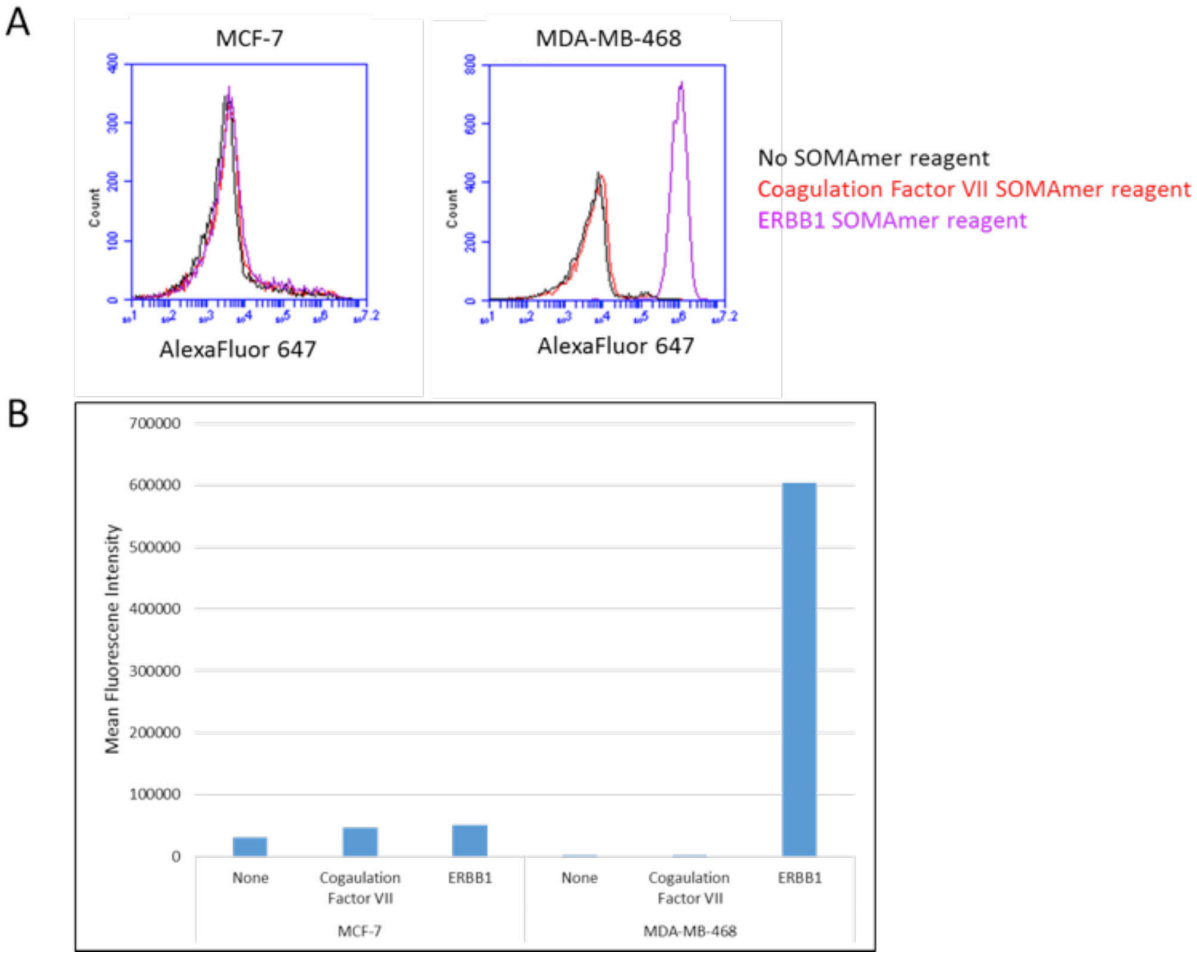

Figure 1. *Detection of ERBB1 using SOMAmer reagents in Flow Cytometry. A) Histogram plots of AlexaFluor 647 intensity illustrate a significant shift in population fluorescence for MDA-MB-468 cells expressing high levels of ERBB1 labeled with an ERBB1-specific SOMAmer reagent. The MCF-7 cell line, which expresses low levels of ERBB1, is not labeled by the ERBB1 SOMAmer reagent. B) Quantitated Mean Fluorescence Intensity for the cell populations presented as histogram plots in panel A.*

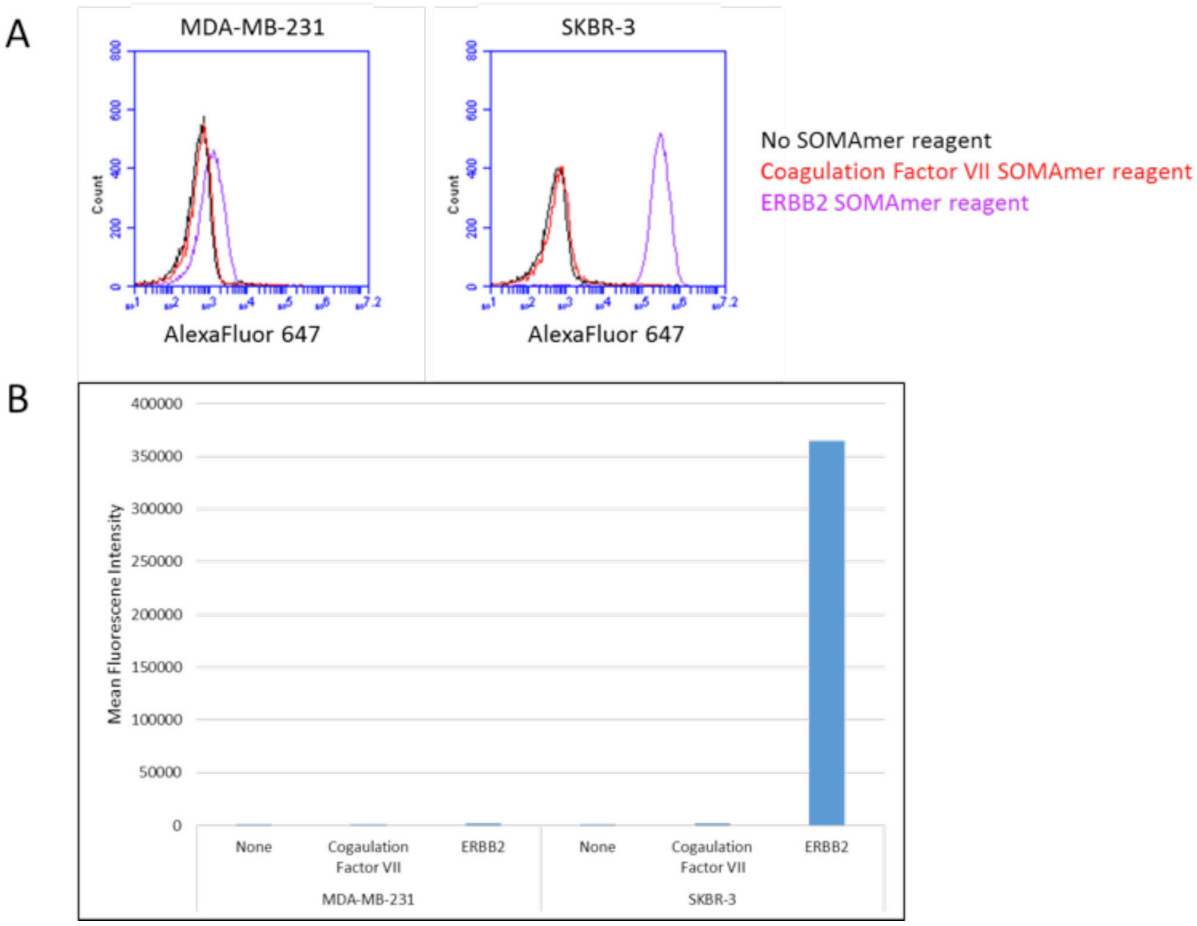

Figure 2. *Detection of ERBB2 using SOMAmer reagents in Flow Cytometry. A) Histogram plots of AlexaFluor 647 intensity illustrate a significant shift in population fluorescence for SKBR-3 cells expressing high levels of ERBB2 labeled with an ERBB2-specific SOMAmer reagent. The MDA-MB-231 cell line, which expresses low levels of ERBB2, is not labeled by the ERBB2 SOMAmer reagent. B) Quantitated Mean Fluorescence Intensity for the cell populations presented as histogram plots in panel A.*

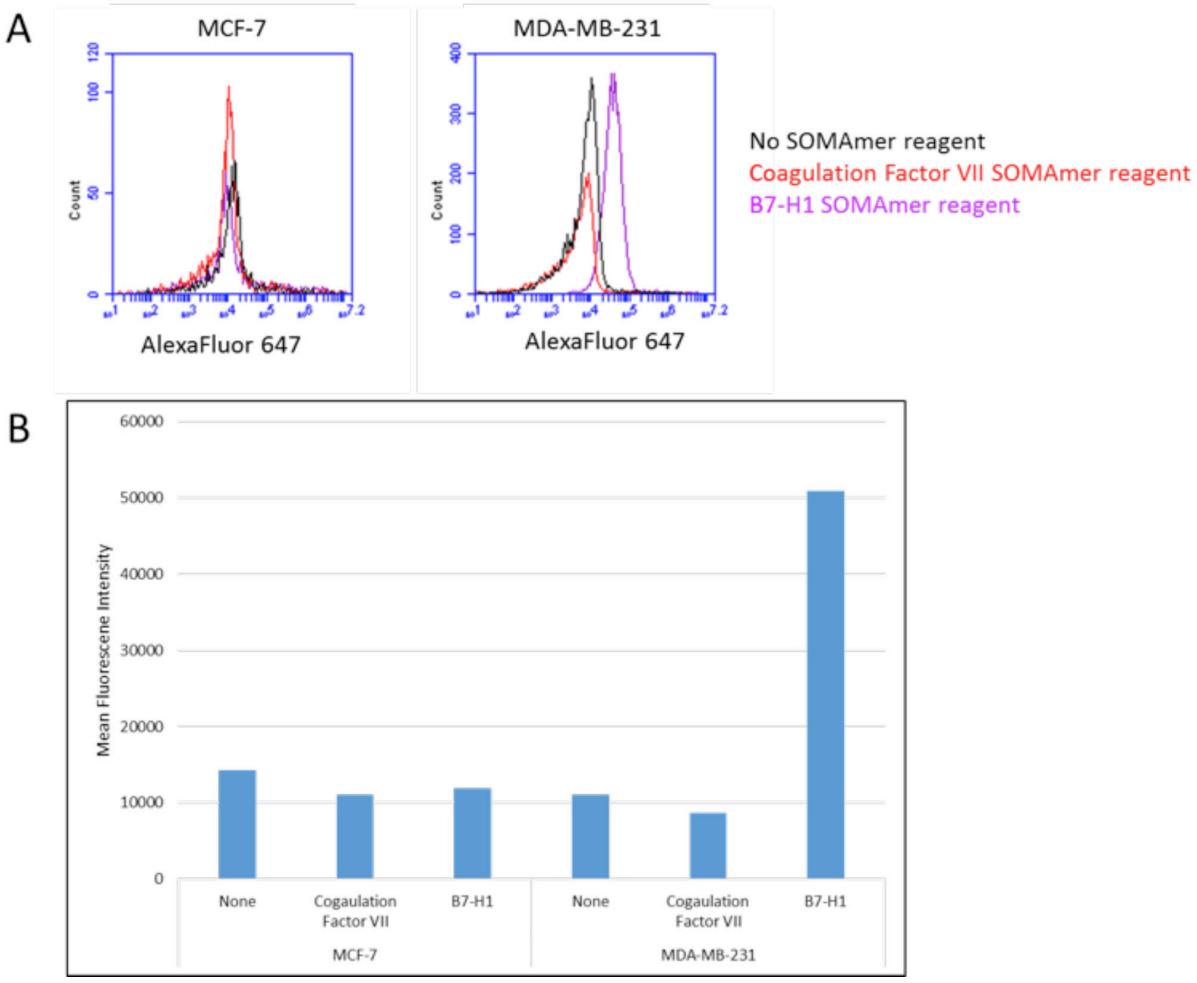

Figure 3. *Detection of B7-H1 using SOMAmer reagents in Flow Cytometry. A) Histogram plots of AlexaFluor 647 intensity illustrate a significant shift in population fluorescence for MDA-MB-231 cells expressing high levels of B7-H1 labeled with a B7-H1-specific SOMAmer reagent. The MCF-7 cell line, which expresses low levels of B7-H1, is not labeled by the B7-H1 SOMAmer reagent. B) Quantitated Mean Fluorescence Intensity for the cell populations presented as histogram plots in panel A.*

References

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DCN 16-064 Rev. 1
AN-15-009 Effective: 3/18/2016

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