Application Note

SOMAmer® Reagent in Flow Cytometry Assays

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. This Application Note discusses using SOMAmer reagents in flow cytometry. **Please note that not all SOMAmer reagents have been tested in all applications.** For help or technical support, please submit your questions by either calling the U.S. Technical Support at 1-800-324-0783, emailing techsupport@somalogic.com or by clicking here to visit the technical support website.

SOMAmer reagents are suitable for detecting and quantifying an extracellular protein by flow cytometry. SOMAmer reagents can easily be adapted to many flow cytometry protocols.

Inclusion of a competitor during or after SOMAmer incubation 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. The SomaLogic® 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.

Recommended Materials

- Cells of interest
- Target-specific biotin-conjugated SOMAmer reagents: 100 µL, 200 nM SOMAmer reagent per cell suspension to be tested
- SomaLogic Buffer with Tween (SBT): 120 mM NaCl, 5 mM KCl, 5 mM MgCl2, 40 mM HEPES pH 7.5, 0.05% Tween® 20 detergent
- FACS Buffer: 120 mM NaCl, 5 mM KCl, 5 mM MgCl2, 40 mM HEPES pH 7.5, 0.01% BSA, 0.15% glucose, pH 7.5
- Plate shaker (optional)
- Centrifuge (e.g., Beckman Coulter Allegra® X-15R Centrifuge, swinging bucket rotor S/N 09T 1312)
- U-bottom 96-well plates (e.g. Thermo Scientific® 96-Well Microtiter Microplates; Catalog No.: 14-245-146)
- Fluorophore-conjugated streptavidin appropriate for flow cytometer (e.g. Pierce™ Streptavidin Protein, FITC conjugate (Thermo Scientific), Life Technologies®, Catalog No. 21224)
- SomaLogic Polyanionic Competitor (SomaLogic, P/N 910-00001)
- Dextran Sulfate (Sigma-Aldrich®, Catalog No.: 31404)

SOMAmer Reagent Preparation

- Thaw SOMAmer reagents and vortex
- Dilute SOMAmer reagents to 1µM in SBT, 150 µL
- Heat SOMAmer reagents to 95°C for 3 minutes, then slowly cool in 25 °C water bath for 20 minutes
- Dilute SOMAmer reagents to 200nM in FACS Buffer

Protocol

1. For cells that grow in suspension, decant the cells into a conical centrifuge tube and perform a cell count and viability analysis and proceed to Step 4
2. For adherent cells, detach your cells from the plate
    - Non-trypsin solutions such as Cell Dissociation Buffer, enzyme-free, Hank's Balanced Salt Solution (e.g., Gibco®; Life Technologies Catalog No.: 13150-016) or 10 mM EDTA in PBS are the preferred method to release attached cells from labware
    - Another common method is scraping but this can result in cell clumps which can clog the cytometer
    - Trypsin solutions are also an option, but can destroy the epitope of the protein you may be interested in staining; you will need to test this empirically
3. Place cells into a conical centrifuge tube and perform a cell count and viability analysis
4. Centrifuge cells to pellet (500 x g, 3 minutes), remove supernatant and resuspend in an appropriate volume of FACS Buffer so that the final cell concentration is 1x106 cells/mL
5. Dispense 100 µL cell suspension (1x105 cells) to wells of U-bottom 96-well plate
6. Dispense 100 µL 200 nM SOMAmer reagent to cells (20 pmoles SOMAmer reagent) and gently mix
7. Incubate 20-60 minutes on ice
8. Wash cells 2 times with FACS Buffer
9. Add 100 µL 2 µg/mL fluorophore-conjugated streptavidin diluted in FACS Buffer
10. Incubate 30 minutes, with gentle shaking
11. Wash cells 2 times with FACS Buffer
12. Resuspend cells in 100-200 µL FACS Buffer
13. Read cells on a flow cytometer

Optimal concentrations of SOMAmer reagent and fluorophore-conjugated streptavidin should be determined experimentally. The addition of 1 µM SomaLogic Polyanionic Competitor and 1µM Dextran Sulfate (Sigma-Aldrich Catalog No.: 31404) during SOMAmer binding may be used to reduce non-specific binding (see SomaLogic Polyanionic Competitor Technical Note). Keep cells on ice until ready to read on a flow cytometer. Cells may be fixed with 1-4% paraformaldehyde in PBS. The optimal concentration of fixative should be determined empirically.

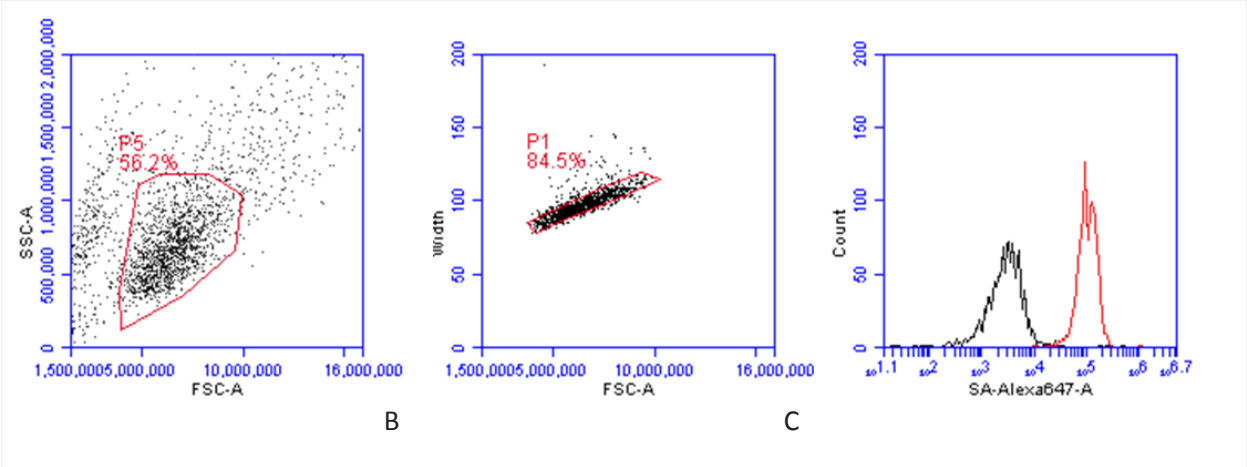

Figure 1. EGF-R detection on MDA-MB-231 cells

Cells were incubated with an EGF-R SOMAmer reagent or a SOMAmer reagent to a non-human protein as described above. Secondary staining was accomplished with Alexa Fluor® 647-streptavidin. Cells (10,000 events) were read on a BD Accuri® C6 flow cytometer. Cells were gated on FSC x SSC to exclude debris (A) and FSC area x FSC width to exclude doublets (B). The EGF-R SOMAmer Reagent is in red and the control SOMAmer reagent is in black (C)

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