SomaLogic

Application Note

# SOMAmer® Reagent Antagonists of Protein Activity

## 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 the suitability of SOMAmer reagents in inhibiting the activities of protein targets. **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 generally bind conformational epitopes across large contact surface areas, and therefore may be capable of inhibiting the activities of protein targets by competing with natural binding partners. Inhibitory SOMAmer reagents can be used as reagents to study biological pathways by specifically decreasing the function of one protein.

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.

## Example 1: SOMAmer Inhibition of IL-6 Activity

Interleukin 6 (IL-6) is a pluripotent cytokine that regulates inflammatory and immune responses and plays a key role in the development of inflammatory diseases (including rheumatoid arthritis) and tumor proliferation. Inhibition of the IL-6 signaling pathway may be an effective approach to manage the progression of these diseases.

SOMAmer reagents that bind human IL-6 were developed at SomaLogic by the SELEX process from a chemically modified DNA library (Gupta et al. 2014). Three SOMAmer reagents exhibited high affinity binding to IL-6 (average Kd = 1 nM) (Figure 1A). A cell-based gene reporter assay was used to evaluate the inhibitory activity of each of the SOMAmer reagents. IL-6 binds to IL-6 receptor and gp130 on the surface of cells and activates the JAK-STAT signaling pathway. HeLa cells were transfected with the pGL3 luciferase reporter vector under control of an IL-6 inducible STAT-responsive element. These cells expressed luciferase upon stimulation with recombinant IL-6, and luciferase expression was reduced with the addition of each IL-6 SOMAmer reagent. A plot of percent luciferase activity relative to the no-SOMAmer control indicated all three SOMAmer reagents reduced IL-6 activation of the JAK-STAT pathway in this assay (Figure 1B).

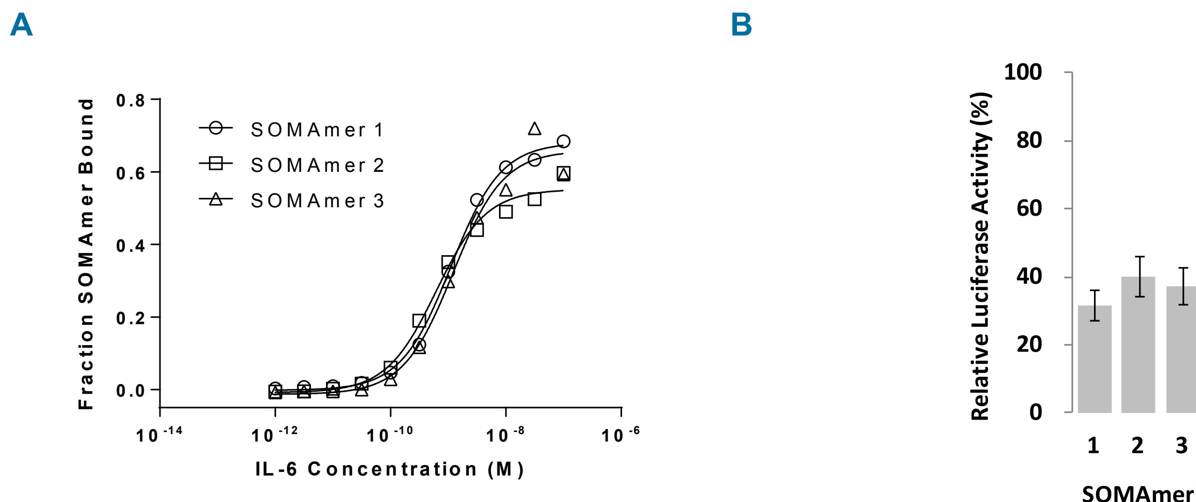


**Figure 1.** Binding and Inhibition Activity of IL-6 SOMAmer reagents. **A.** Binding activity of three IL-6 SOMAmer reagents discovered with the SELEX process. **B.** Inhibition activity of three IL-6 SOMAmer reagents in a cell-based assay. The mean and standard deviation of triplicate measurements are plotted. IL-6 (10 ng/mL) was applied to cultured cells with or without SOMAmer reagent (1 µM) for 24 hours and luciferase activity was measured.

## Recommended Materials

- Recombinant human IL-6 (e.g. R&D Systems, Catalog #206-IL-050/CF)
- StrataCleanTM resin (e.g. Agilent Technologies®, Catalog #400714 )
- MultiScreen®-HV Filter Plate (e.g. EMD Millipore®, Catalog #MAHVN4550)
- Vacuum and manifold for filtration of 96-well plates
- SBT: 120 mM NaCl, 5 mM KCl, 5 mM MgCl2, 40 mM HEPES pH 7.5, 0.05% Tween® 20 detergent
- Phosphorimager
- HeLa cells with vector containing luciferase gene under control of STAT-responsive element
- Luciferase substrate
- Cell culture incubator
- Plate reader
- γ-32P-dATP
- T4 polynucleotide kinase

## Measurement of Binding Affinity ($K_d$)

1. Radiolabel SOMAmer reagent with 32P using γ-32P-dATP and T4 polynucleotide kinase
2. Incubate SOMAmer reagent (20 pM) with IL-6 (1 pM - 100 nM) in SBT for 30 minutes at 37°C
3. Add StrataClean resin (see manufacturer's instructions) and gently shake for 30 seconds
4. Pass solution through MultiScreen-HV filter plate membrane under vacuum and wash with 200 μL SBT
5. Remove plastic underdrain and position plate on phosphor screen
6. Scan phosphor screen on phosphorimager and quantify signal in each well
7. Plot signal as a function of IL-6 concentration and calculate Kd using a 3-parameter fit

## Evaluation of SOMAmer Inhibition Activity

1. Plate transfected HeLa cells in DMEM containing 10% FBS at 5 x 104 cells/well
2. Culture for 1 day at 37°C in a CO2 incubator
3. Equilibrate IL-6 (10 ng/mL) with or without SOMAmer reagent (30 pM - 100 nM) in SBT for 30 minutes at 37°C
4. Add IL-6/SOMAmer mixture to cells and incubate for 1 day at 37°C in a CO2 incubator
5. Discard supernatant, add luciferase substrate reagent and incubate at ambient temperature for 30 minutes
6. Quantify luminescence using a plate reader

## References:

Gupta et al. 2014, Chemically Modified DNA Aptamers Bind Interleukin-6 with High Affinity and Inhibit Signaling by Blocking its Interaction with Interleukin-6 Receptor. J Biol. Chem. 289: 8706.

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