Application Note

Polyanionic Competition

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. **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.

Inclusion of a competitor during or after incubation of the reagent with a sample can be valuable in distinguishing between specific and non-specific binding in all SOMAmer reagent applications. The example below illustrates the use of a SomaLogic® Polyanionic Competitor (P/N 910-00001) as a universal SOMAmer reagent competitor. 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. The SomaLogic Polyanionic Competitor at concentrations ranging from 0.1 µM to 10 mM can be evaluated for use in specific applications.

Example of use: TGF-β1 binding to TGF-β SOMAmer Reagents

Non-specific binding of SOMAmer reagents can often occur in the presence of closely related proteins and can be compounded by a protein with a basic pI. For example, the TGF-β proteins are structurally similar; TGF-β1 is 71% and 77% identical to TGF-β2 and TGF-β3, respectively. Additionally, the TGF-β1 protein has a basic pH(I) of 8.6 which can increase its affinity for random DNA. The binding of the TGF-β1 protein to TGF-β1, TGF-β2 and TGF-β3 SOMAmer reagents was measured both in the absence of an up-front competitor and with the addition of 1 µM SomaLogic Polyanionic Competitor. As can be seen in the binding curves in Figure 1, TGF-β1 bound to the TGF-β1 SOMAmer reagent, though there was also cross-reactive binding, albeit with lower affinity, to TGF-β2 and TGF-β3 SOMAmer reagents as well. Binding to a random library was also observed. Addition of 1 µM SomaLogic Polyanionic Competitor represses the binding to random DNA, as well as to the TGF-β2 reagent, in the concentration range measured (1 pM to 100 nM). Binding to the TGF-β3 reagent is reduced 35X upon addition of the competitor. Nonetheless, the affinity of TGF-β1 for its own SOMAmer reagent is nearly two logs better than that for the TGF-β3 reagent.

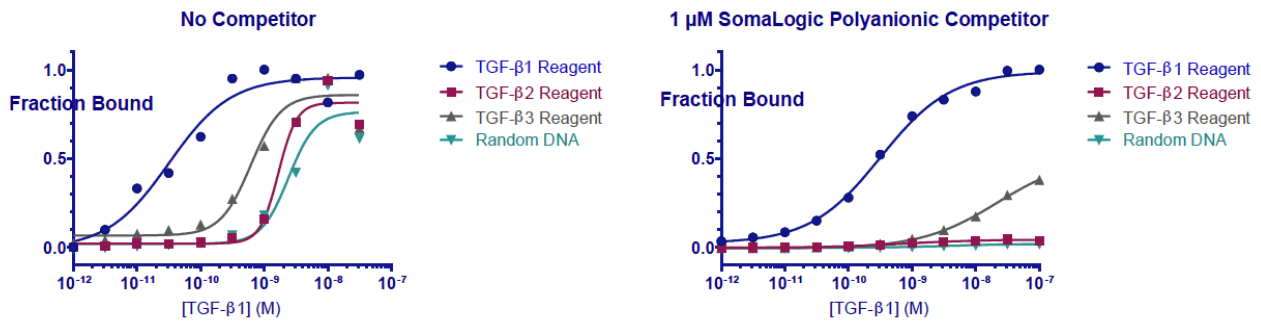

Figure 1: Binding curves for TGF-β1 binding to SOMAmer reagents selected against TGF-β family members without (left) and with (right) 1 µM SomaLogic Polyanionic Competitor. Random DNA binding is significantly diminished, while specific binding is largely unaffected, by the SomaLogic Polyanionic Competitor.

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