Application Note

SOMAmer® Reagent Capture for Mass Spectrometry and SDS Page

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.

This Application Note discusses using SOMAmer reagents in mass spectrometry and SDS Page. For a SOMAmer reagent capture assay, SOMAmer reagents are pre-immobilized on streptavidin agarose beads and incubated with purified protein and/or matrix. The addition of DNA competitors in the incubation reaction and a dextran sulfate wash of the SOMAmer-protein complexes will reduce low level binding of highly abundant non-specific proteins. Captured proteins may be pre-stained with a NHS-fluorophore, such as Alexa Fluor® 647, to visualize the pull-down protein(s) on a gel for post-assay analysis.

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.

When processing samples for mass spectrometry, a replicate sample is run with no Alexa Fluor 647 stain. Captured protein may be released from the SOMAmer-protein complex with 20mM sodium hydroxide or digested on the beads for direct mass spectrometry analysis. If needed, the Tween® 20 concentration in the final elution buffer may be lowered (0.01% or less) for mass spectrometric samples.

SOMAmer Reagent Preparation

- Thaw SOMAmer reagents and vortex
- Dilute SOMAmer reagents to 50nM in SBT (defined below in Buffers), 100µL
- Heat SOMAmer reagents to 95°C for 3 minutes, then slow cool in 25°C water bath

Protein/Sample Preparation

- 0.1µM protein in SBT, 50µL ± DNA competitor
- 1%-40% plasma in SBT, 50µL depending on target abundance, ±DNA competitor
- Other samples (e.g. cell lysates, cell supernatants), ±DNA competitor

Buffers

1. SBT: 40mM HEPES pH 7.5, 102mM NaCl, 1mM EDTA, 5mM MgCl2, 5mM KCl, 0.05% Tween20 detergent
2. CAPS Buffer: 50mM CAPS, 1mM EDTA, 0.05% Tween 20, pH 11.0
3. Dextran Sulfate Buffer: 10mM dextran sulfate/ SBT
4. Staining Buffer: 0.1mM Alexa Fluor 647 NHS Ester/SBT
5. Glycine Buffer: 20mM glycine/SBT, pH 7.5
6. 20mM NaOH (sodium hydroxide)
7. 80mM HCl (hydrochloric acid)

Recommended Supplies and Instrumentation

1. Thermomixer® or plate shaker
2. MultiScreen® HV 96-well filter (e.g. EMD Millipore®; Catalog No.: MAHVN4550)
3. Fisherbrand™ 96-Well DeepWell™ (well capacity of 1mL) Plate (Fisher Scientific®; Catalog No.: 12-566-120)
4. Light source, UV 360nm
5. Vacuum manifold, vacuum filtration station
6. Centrifuge with swinging bucket and rotor that holds deep-well plates
7. Steptavidin-Agarose (e.g. Thermo Scientific™; Catalog No.: 20353)
8. Alexa™ Fluor 647 NHS Ester (Succinimidyl Ester), Life Technologies®; Catalog no.: A-20006
9. Dextran sulfate sodium salt from Leuconostoc spp. (Sigma-Aldrich®; Catalog No.: 31404)
10. DNA Competitors: herring sperm DNA, tRNA or SomaLogic Polyanionic Competitor (Product number 910-00001)

Example SOMAmer Reagent Affinity Capture Protocol

SOMAmer Reagent Immobilization on Steptavidin Agarose Beads

1. Pre-wet all wells of a MultiScreen HV 96-well filter plate with 50µL SBT, place over vacuum manifold and apply gentle vacuum to remove buffer
2. Add 133µL 7.5% streptavidin agarose beads (diluted in SBT, the initial 50% slurry needs no additional treatment) to pull-down sample wells of filter plate, apply vacuum
3. Wash beads 2x 190µL SBT, vacuum filter between washes
4. Immediately add SOMAmer reagent to streptavidin agarose beads, mixing gently to resuspend beads
5. Incubate 20 min with shaking (850 rpm), at 25°C
6. Apply vacuum to filter plate
7. Wash 2x 190µL CAPS Buffer, with 1 minute shaking (850 rpm), vacuum between washes
8. Wash 2x 190µL SBT, with 1 minute shaking (800 rpm)

Protein-SOMAmer Reagent Incubation

1. Apply vacuum and blot bottom of filter plate, immediately add 50µL of the prepared sample to the SOMAmer reagent-immobilized beads, mixing gently to resuspend beads
2. Cover plate (press lightly along plate edges)
3. Incubate a minimum of 1.5 hours with shaking (800 rpm), at 28°C
4. After equilibration, vacuum filter plate and add 100µL dextran sulfate buffer to samples
5. Incubate shaking (800 rpm) for 5 minutes at room temperature, vacuum filter plate
6. Wash 6x 190µL SBT, vacuum filter between washes, leave last wash on beads

Alexa Fluor 647 Labeling (if required)

1. Prepare staining buffer (use within 15 minutes of preparation)
2. Apply vacuum to plate and add 50µL staining buffer to samples requiring AlexaFluor 647 labeling
3. Add 50µL SBT to un-labeled samples
4. Shake 10 minutes (800rpm) at room temperature
5. Wash 2x glycine buffer, vacuum filter between washes
6. Wash 6x 190µL SBT, vacuum filter between washes

Release of SOMAmer reagent captured protein with NaOH

1. Remove final SBT wash by vacuum filtration and blot plates
2. Remove skirt from filter plate and place plate over a 1mL deep well plate
3. Add 40µL 20mM NaOH to each sample well, mix gently
4. Incubate at room temperature for 2 minutes.
5. Centrifuge samples into deep well plate, 1000xg, 1 minutes (swinging bucket)
6. Add 10µL 80mM HCl to 40µL each sample well to neutralize samples to pH ~7

Sample gel analysis

Pre-labeled Alexa Fluor 647 samples

10µL of a SOMAmer reagent captured sample is usually adequate for imaging the Alexa Fluor 647-stained proteins on a gel.

Purified protein markers may be prepared by adding 1µL 0.1mM staining buffer to 50nM protein/ 100µL SBT. Proteins must be free of any amine-containing substances such as tris, glycine, ammonium ions, or stabilizing proteins such as bovine serum albumin.

Instructions below are for NuPAGE® Novex® gel analysis. Make any appropriate changes to fit your protein gel protocol.

NuPAGE Novex 10% Bis-Tris Protein Gel (Life Technologies; Catalog No.: NP0327BOX)

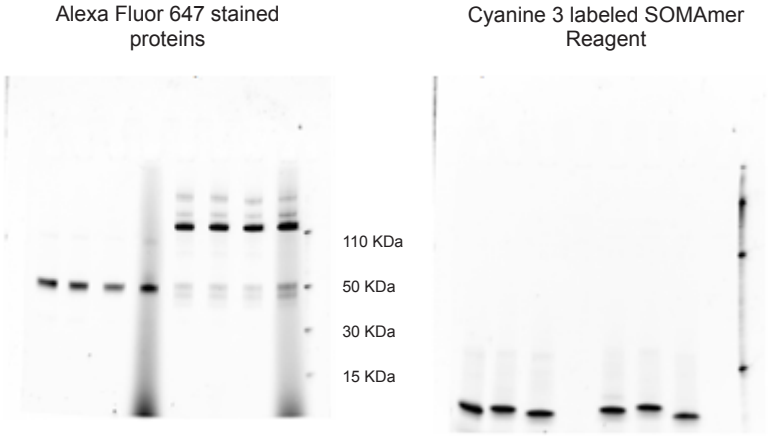
Alexa Fluor 647-stained sample	10µL
4X NuPAGE sample buffer + 50mM DTT	3.3µL

Markers: Novex Sharp Pre-stained Protein Standards (Life Technologies; Catalog No.: LC5800). The 4 blue bands fluoresce under cyanine 5 settings.

Gel Protocol

1. Heat gel samples at 70°C for 10 minutes
2. Load entire sample on gel, run at 200 volts for 45 minutes, until bromophenol blue runs off the gel

Image gel fluorescence: excitation 650nm, emission 668nm to visualize Alexa Fluor 647-stained protein(s), excitation 530nm/ emission 580nm to visualize the SOMAmer Cyanine 3 label.



Lane	SOMAmer reagent captured samples
1	SOMAmer 1 – Target 1
2	SOMAmer 2 – Target 1
3	SOMAmer 3 – Target 1
4	Target 1
5	SOMAmer 1 – Target 2
6	SOMAmer 2 – Target 2
7	SOMAmer 3 – Target 2
8	Target 2
9	Novex pre-stained markers

Figure 1: SOMAmer reagent capture of 2 proteins, each with 3 protein-specific SOMAmer reagents.

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