Application Note

Antibody-SOMAmer® Sandwich Assay

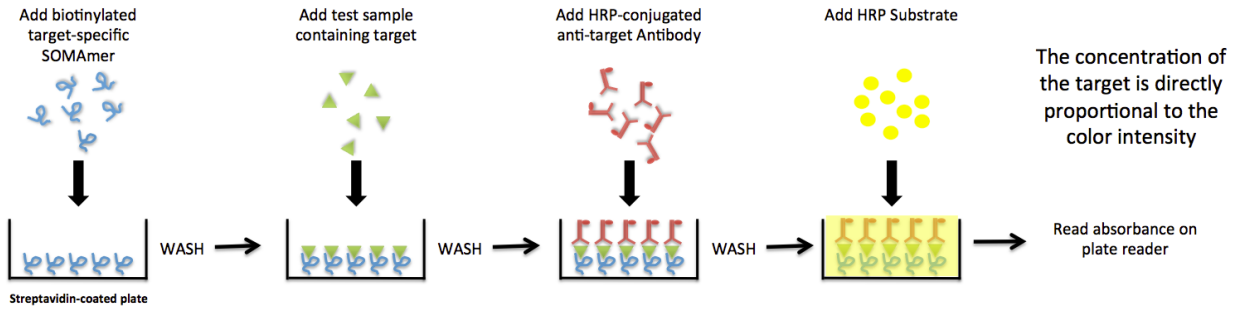
Introduction

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.

The protocols given below illustrate the use of SOMAmer reagents as capture and detection reagents. Either method can be done on plates or on beads.

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.

SOMAmer protein-capture reagents in a SOMAmer-antibody sandwich ELISA assay



Materials

- Target protein
- Target-specific HRP-conjugated antibody
- Target-specific biotin-conjugated SOMAmer reagents
- Streptavidin Coated Plates (Thermo Scientific™ 15124)
- SBT buffer: 120mM NaCl, 5mM KCl, 5mM MgCl2, 40mM HEPES pH 7.5, 0.05% Tween20
- TBST buffer: 150mM NaCl, 20mM Tris, pH 7.6, 0.05% Tween20
- Streptavidin blocking buffer: 100µM biotin/ SBT
- Plate blocking buffer: 3% BSA/ SBT buffer
- HRP Substrate (TMB 3,3',5,5'-tetramethylbenzidine) (Thermo Scientific P/N 34028)
- 2M Sulfuric acid
- Plate shaker
- Spectrophotometer plate reader

SOMAmer reagent immobilization on streptavidin plate

1. Dilute biotin-conjugated SOMAmer reagent to 200nM in SBT, 100µL. Heat-cool at 95°C, 3 minutes then slow cool in 25°C water bath for 20 minutes.
2. Dilute SOMAmer reagents to 20nM, final volume 1mL
3. Wash the Reacti-Bind™ streptavidin coated plate 3X, 200µL SBT (dump and tap)
4. Add 100µL diluted SOMAmer reagent to 8-wells each. Seal plate and incubate 4 °C, overnight
5. Wash wells 3X, 300µL SBT
6. Block wells with 200µL streptavidin blocking buffer, 10 minutes, room temperature
7. Wash 3X 300µL SBT, 1 minute, 100 rpm
8. Block with 300µL Blocking buffer, 30 minutes, room temperature
9. Wash 3X 300µL SBT, 1 minutes, 100 rpm

SOMAmer Reagent-Target capture

1. Dilute target protein to 10nM in SBT, 200µL
2. Prepare ½ log serial dilutions of protein in SBT
3. Transfer 100µL protein dilution to SOMAmer coated wells
4. Incubate 37 °C, 450 rpm
5. Wash 3X 300µL TBST, 1 minute, 100 rpm

SOMAmer Reagent-Target Detection

1. Dilute HRP-conjugated antibody in 3% BSA-TBST to the recommended dilution
2. Add 100µL antibody to wells, incubate 30 minutes, room temperature, 450 rpm
3. Wash 3X 300µL TBST, 1 minute, 100 rpm
4. Add 100µL TMB substrate to wells. After 8-12 minutes, add 50µL 2M sulfuric acid to stop color development
5. Read absorbance 450nm

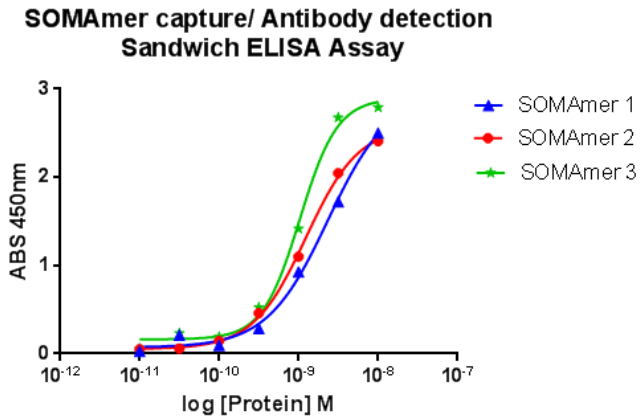
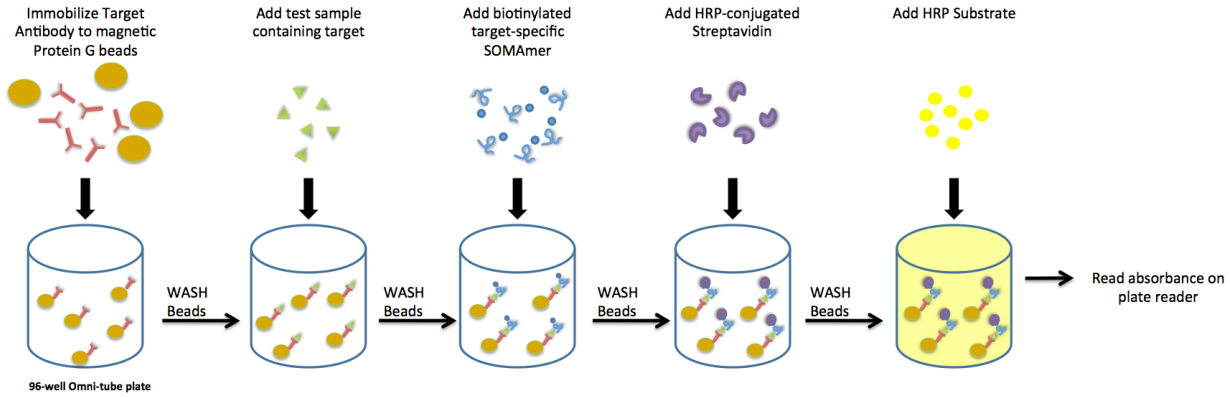


Figure 1. Three SOMAmer reagents were found to pair with a detection antibody in a sandwich ELISA assay. The capture SOMAmer reagents were immobilized on streptavidin plates and incubated with serial dilutions of protein. An HRP-conjugated antibody was added for detection of the target.

SOMAmers protein-detection reagents in a SOMAmer-antibody sandwich ELISA assay



Materials

- Target protein
- Target-specific antibody
- Target-specific biotinylated SOMAmer reagents
- SBT buffer: 120mM NaCl, 5mM KCl, 5mM MgCl2, 40mM HEPES, 0.05% Tween20
- Dynal® magnetic Protein G beads (Life Technologies® 100-09D)
- Omni-tube 96-well microtiter plates (Thermo Scientific NC0508658)
- Streptavidin-Horseradish Peroxidase (SA-HRP) (Life Technologies S-911)
- TMB (3,3',5,5'-tetramethylbenzidine) Substrate (Thermo Scientific 34028)
- 2M sulfuric acid
- Bead magnet for 96-well plate and individual 1.7mL microcentrifuge tubes
- Plate shaker
- Spectrophotometer plate reader

Antibody-capture bead preparation

10. Transfer 1 mg Protein G beads (33µL, 30mg/ml) to 1.5ml microcentrifuge tube. Place on magnet and remove supernatant. Wash beads 3X with 100µL SBT
11. Dilute target-specific antibody 0.1 mg/ml in 70µL SBT and add to beads. Resuspend beads and shake for 20 minutes, 37C
12. Wash beads 3X, 100µL SBT
13. Resuspend beads to 2.5 mg/ml in SBT, 400µL

Target capture on antibody beads

6. Dilute target protein to 10nM in SBT, 250µL
7. Prepare 1:2 serial dilutions of protein to 10pM
8. Transfer 100uL of each protein dilution to wells of Omni-tube microtiter plate
9. Add 25µL antibody-bead suspension to each protein dilution
10. Gently shake, keeping beads in suspension, 30 minutes, 25°C
11. Wash bead 3X, 100µL SBT

SOMAmer Reagent Target Detection

6. Dilute SOMamer reagent to 0.3µM in 100µL SBT. Heat 95°C, 3 minutes, then slow cool to 25°C in water bath. Place at room temperature
7. Dilute SOMAmer reagents to 20nM with SBT (1400uL)
8. Add 100µL SOMAmer reagent to target-antibody-beads
9. Shake beads 1 hour, 25°C
10. Wash beads 3X 100µL SBT
11. Remove last wash, resuspend beads in 80µL SA-HRP (2µg/mL in SBT), shake 30 minutes, 25°C.
12. Wash beads 2X 100µL SBT
13. Transfer beads to new microtiter plate with 3rd 100µL SBT wash
14. Add 100µL TMB substrate, shake beads. After 1-5 minutes, add 50µL 2M sulfuric acid to stop color development
15. Place samples on magnet. Transfer 100µL to 96-well plate. Read absorbance at 450nm

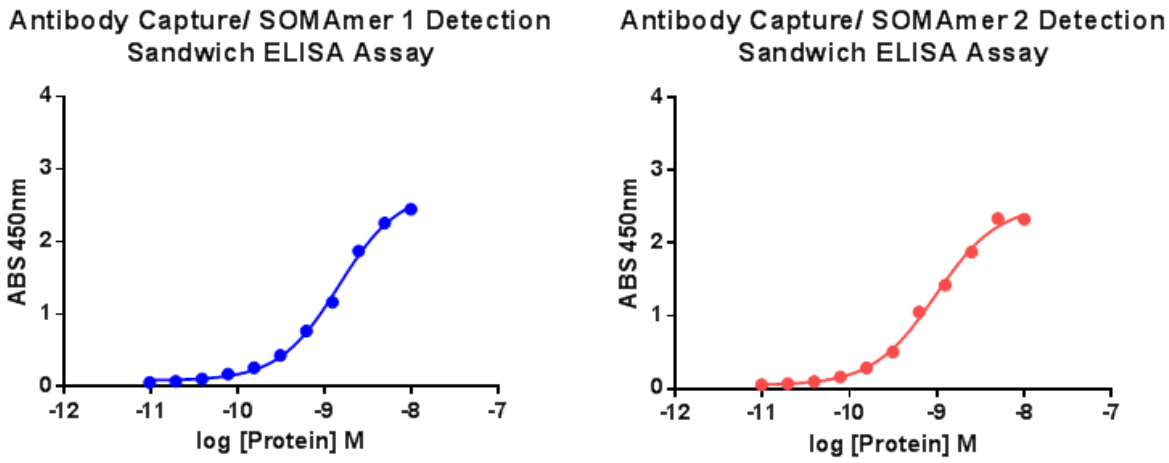


Figure 2. Protein G- antibody beads captured serial dilutions of protein in solution. SOMAmer 1 and SOMAmer 2 were both able to act as detection reagents in the sandwich ELISA assay.

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