SOMAmer® Reagents: Specific and Versatile Protein Affinity Reagents

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• Founded in 2000
• Built upon DNA aptamer technology developed in Larry Gold’s lab at CU Boulder
• Modified aptamers are used as protein binding and detection reagents in a multiplexed proteomic assay
• SomaScan Biomarker discovery tool measures 1310 proteins across 8 orders of magnitude in a small biological sample (150 µl of plasma or serum)
• High throughput capability of thousands of samples per week
• A subset of these 1310 reagents are being offered individually for the life science researcher
SELEX: SOMAmer Reagent Discovery Method
Systematic Evolution Of Ligands By EXponential Enrichment

Start:
Library of sequences \( (10^{15}) \) + Target protein

Sequences are used in the next round of selection or sequenced for screening

Sequences are amplified

Bound sequences released from target

Protein target and bound sequences captured on magnetic beads

Unbound sequences washed away
Chemically synthesized modified DNA

DNA modifications result in:
- Increased chemical diversity
- High affinity (sub-nM)
- Unique binding profiles enabled by hydrophobic interactions

Current public menu of 277 SOMAmer Reagents

Protein targets with known roles in oncology, immune oncology, AD, immune regulation, neurobiology, etc.

Custom selections available for unique targets, desired binding properties

A variety of 5’ functional groups are possible (e.g., fluorophores, biotin, amine, azide, PEG)

Available only from SomaLogic
Modifications to Nucleic Acids

- Incorporation of protein-like side-chains, examples above, increases physico-chemical diversity
- Modified libraries yield SOMAmer reagents with high affinities (sub-nM median) and slow off-rates
- The use of modified nucleotides in the **SELEX** process significantly increases the success rate of new SOMAmer discovery compared to unmodified aptamers

See also:

SOMAmer Reagents Can Be Developed to Proteins With A Broad Range Of Properties

- Reagent affinities for protein analytes are typically $\leq 1 \text{ nM}$
- $t_{1/2}$ for cognate complexes are typically $> 30 \text{ minutes}$
- SOMAmer reagents have been discovered for acidic and basic proteins, intracellular, membrane bound, and extracellular
SOMAmer Reagents Currently Available

• 277 SOMAmer reagents, each targeting unique protein

• For Research Use Only (RUO)

• Selected against human proteins
  – Binding to non-human primate and rodent homologs has been observed but formally tested for all commercially available SOMAmer Reagents

• 500 pmol / tube (~10 μg)
  • 10 μM solution of SOMAmer reagent in 5 mM HEPES, 1 mM EDTA, pH8
  – SomaLogic Polyanionic Competitor is recommended for many applications
    • 1 tube of Competitor is provided per 1 tube of SOMAmer reagent
    • Provided at 10x the concentration of SOMAmer
  – Additional reagents to be added as they are qualified and manufactured

• Countries: US, Canada, EU and Switzerland
SOMAmer® Reagents:
Specific and Versatile Protein Affinity Reagents

SPECIFICITY
CHARACTERIZATION
Testing Binding to Related Proteins

• Proteins often exist in “families” with varying degrees of amino acid sequence identity and structural homology

• Proteins must share >40% sequence identity with the SELEX target protein across a significant stretch

• Must be in SomaLogic protein inventory or commercially available

• Other considerations:
  – Shared subunits
  – Proteolytic cleavage products

Method:
• SOMAmer Reagent pulldown assay with purified protein
• Analysis by SDS-PAGE
Highly Specific Protein Target Binding

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**CA I Capture Reagent**

- CA I
- CA I standard
- CA Xll
- CA Xll standard
- CA II
- CA II standard
- CA III standard
- CA III
- CA VII standard
- CA VII
- CA X standard
- CA X
- CA IV standard
- CA IV
- CA VII standard
- CA VII
- MW markers

**IL-17 Capture Reagent**

- IL-17
- IL-17 standard
- IL-17F
- IL-17F standard
- IL-17B
- IL-17B standard
- IL-17D
- IL-17D standard
- MW markers

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10
Cross-reactive with disparate affinities

ERBB1 Capture Reagent

ERBB1 standard
ERBB2 standard
ERBB3 standard
ERBB4 standard
MW markers

ERBB1

Legend

Synthetic SOMAmers
SOMAmers: SOMAmers 2677-1, 2677-3, Protein G

$K_D = 0.1 \text{ nM}$

ERBB3

Legend

Synthetic SOMAmers
SOMAmers: SOMAmers 2677-1, 2677-3, Protein G

$K_D = 5 \text{ nM}$
SOMAmer Reagents Product Information

SOMAmer anti-Carbonic anhydrase 1 B-4969-2_1

Product # 910-00226

Your Price $390.00
Quantity 1

ADD TO CART

Description

SOMAmer Reagent 4969-2_1
Protein Target Carbonic anhydrase 1
Target UniProt ID P00915
Species Reactivity Human
Amount 500 pmol
Composition Biotinylated Synthetic Oligonucleotides
Target Source R&D Systems®, 2160-CA-050
Formulation Buffered SOMAmer reagent delivered at 10 μM in 5 mM HEPES, 1 mM EDTA, pH 8

Activity

SOMAmer reagents have been qualified for target affinity capture in conjunction with numerous downstream applications. Specific protocols should be determined for the intended use. General application notes are available on our website. (http://www.somalogic.com/Resources/App-notes.aspx)

Specificity

No binding was observed Carbonic anhydrase II, Carbonic anhydrase III, Carbonic Anhydrase IV, Carbonic anhydrase 6, Carbonic anhydrase VII, Carbonic Anhydrase X, or Carbonic anhydrase XIII.

Affinity

The Kd for Carbonic anhydrase 1 binding to the SOMAmer reagent, measured by His Alpha method, is typically 1x10^-9 M. See Certificate of Analysis for details.
SOMAmer® Reagents:
Specific and Versatile Protein Affinity Reagents

Life Science Research Applications
SOMAmer Reagents Enable Experiments on Many Standard Laboratory Instruments

- **ELISA**
  - Ligand Binding Assays
  - Customizable panels
  - Validated assays

- **Mass Spectrometry**
  - Analyte-enrichment
  - Common contaminant depletion
  - Protein complex identification

- **Flow Cytometry**
  - Multiplex cell surface & intracellular proteins
  - Compatible with multiple label molecules

- **Histology & Cell Microscopy**
  - Imaging of native proteins *in situ*
  - Compatible with multiple label molecules
Sandwich Assays with SOMAmer Reagents

- SOMAmer reagents can be used in conjunction with another SOMAmer reagent or an antibody to perform sandwich assays.

- See also:
Sandwich Assays with SOMAmer Reagents

1. Add biotinylated target-specific SOMAmer reagent
2. Add test sample containing target
3. Add HRP-conjugated anti-target Antibody
4. Add HRP Substrate

The concentration of the target is directly proportional to the color intensity

Read absorbance on plate reader
SOMAmer Reagents Function as Sandwich Capture or Detection Reagents
Fluorescent Cell Microscopy

Fix: 4% Formaldehyde, 10’ R/T

Traditional Ab-based SOMAmer Reagents

Primary Detection

Secondary Detection
Fluorescent Cell Microscopy

Coagulation Factor VII SOMAmer reagent

SKBR3
ERBB1^{Low}
ERBB2^{High}

ERBB1 SOMAmer reagent

MDA-MB-468
ERBB1^{High}
ERBB2^{Low}

ERBB2 SOMAmer reagent

MDA-MB-231
ERBB1^{Low}
ERBB2^{Low}
Flow Cytometry Overview
Flow Cytometry with SOMAmer Reagents

ERBB2

No SOMAmer reagent
Coagulation Factor VII SOMAmer reagent
ERBB2 SOMAmer reagent

EGFR / ERBB1

No SOMAmer reagent
Coagulation Factor VII SOMAmer reagent
ERBB1 SOMAmer reagent

PD-L1

No SOMAmer reagent
Coagulation Factor VII SOMAmer reagent
PD-L1 SOMAmer reagent
SOMAmer Reagents Used as a Histochemical Probe of ERBB2 in Breast Cancer Tissue

SOMAmer® Reagents:
Specific and Versatile Protein Affinity Reagents

Experimental Design with SOMAmer Reagents
Aptamers are not antibodies
Proteins with inherent positive surface charge will have some degree of attraction to negatively charged molecules (i.e. SOMAmer Reagents and other nucleic acids).

Blocking these sites with a polyanionic competitor (10x molar excess over SOMAmer Reagent) greatly increases specific binding to protein epitope.

Common polyanionic competitors: Dextran sulfate, sheared herring sperm DNA, SomaLogic Polyanionic Competitor (provided with reagents)

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**Polyanionic Competitor Reduces Non-Specific Binding**

![Graphs showing fraction bound vs. [TGF-β1] (M) with and without competitor](image-url)
Polyanionic Competitor Reduces Non-Specific Binding

100 nM ERBB2 Cy3-SOMAmer Reagent
DAPI

100 nM ERBB2 Cy3-SOMAmer Reagent
DAPI
1 mM Dextran Sulfate, 30 minutes
Experimental Considerations: Protein Target

- **Native vs. denatured**
  - All reagents are selected to bind native, folded protein in solution
  - Unlikely to perform in Western blots

- **Species of origin**
  - All reagents are selected against human protein
  - Binding has been observed with rodent and non-human primate homologs, not formally tested for all reagents

- **Analyte concentration in sample**
  - Solution $K_D$ is reported for each SOMAmer Reagent measured against purified protein target
Experimental Considerations: Mode of Detection

• 5’ Biotin on all currently available SOMAmer reagents

• Common Streptavidin conjugates:
  – For Protein Detection:
    • Horseradish peroxidase (ELISA)
    • Alkaline phosphatase (ELISA)
    • Fluorophores (IHC, Cell Microscopy, Flow Cytometry)
    • Gold nanoparticles
  – For Protein Capture:
    • Agarose or magnetic beads
    • Coated ELISA plates

• Consider pre-binding streptavidin conjugate to reduce biotin-dependent background in complex samples

• Alternative 5’ functionalities are available: Amine, Azide, fluorophore, PEG, etc.
Experimental Considerations: Sample Composition and Complexity

• Milieu of protein target
  – Purified protein in buffer
  – Engineered overexpression cell line or cell lysate
    • Block binding to positively charged proteins
  – Plasma, serum, cell lysate or other biological matrix
    • Analyte concentration
    • Block binding to positively charged proteins
  – Tissue section or cell line
    • Extent and mode of fixation (denaturing, extent of cross-linking)
    • Fresh frozen vs. Formalin-fixed
    • Intact or permeabilized (block binding to genomic DNA and proteins)
What About Antibodies?

SOMAmer® Reagents:
Specific and Versatile Protein Affinity Reagents
SOMAmer Reagents vs. Antibodies

- Selected against native protein
- Need to block DNA-protein interactions (polyanionic competitors)
- “Monoclonal” i.e. single sequence
- Produced synthetically
- Lot-to-lot consistent and scalable manufacturability
- High thermal stability

- Raised against native, denatured protein, or linear peptide
- Need to block protein-protein interactions (BSA, milk, serum)
- Monoclonal or polyclonal
- Produced in vivo, in hybridoma, recombinant expression
- Variable consistency and scalability
- Variable thermal stability
Issues with Antibodies

• There are well-documented challenges with research reproducibility in experiments using antibodies

• Variable quality and stability between lots (assay deployment lifetime?)

• Limited specificity characterization

• Varied degrees of antigen description (native, denatured, peptide?)

Further Reading:


Aptamers are not antibodies
Summary

• Unique hydrophobic modifications differentiate SOMAmer Reagents from traditional DNA aptamers

• SOMAmer Reagents are well characterized, robust and reproducible protein affinity reagents

• SOMAmer Reagents can be used in many common protein detection assays
  – ELISA, FACS, IHC, Cell Microscopy, enrichment for MS, affinity enrichment or depletion
Web Resources

SOMAmer Reagents e-store:
http://estore.somalogic.com

Application Notes
http://www.somalogic.com/Resources/App-Notes

FAQs
http://www.somalogic.com/Products-Services/FAQ

SOMAmer Discovery Services
http://www.somalogic.com/Products-Services/SOMAmer-Discovery-Service

SomaScan Assay
http://www.somalogic.com/Products-Services/SOMAscan
SOMAmer Reagents Used As Histochemical Probes – Macrophages in Lung Tissue

- SOMAmer to macrophage mannose receptor (orange, highlights alveolar macrophages)
- Antibody to cytokeratin (green, highlights lung epithelium)
- Antibody to CD31/PECAM-1 (red, highlights blood vessels)
- DAPI (blue, highlights DNA in nuclei)
Considerations when developing a protein affinity assay

• Status of protein to be detected
  – Native vs. folded
  – Species of origin
  – Concentration in sample

• Mode of detection

• Milieu of protein target
  – Purified protein in buffer
  – Fixed tissue or cell line
  – Plasma, serum or other complex matrix
Cross-reactive with similar affinities
# SOMAmer Reagents Combine The Best Properties of Antibodies and Traditional Aptamers

<table>
<thead>
<tr>
<th></th>
<th>Antibodies</th>
<th>Aptamers</th>
<th>SOMAmer Reagents</th>
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</thead>
<tbody>
<tr>
<td><strong>Discovery success rate</strong></td>
<td>~90%</td>
<td>~30%</td>
<td>~85%</td>
</tr>
<tr>
<td><strong>Affinity for target proteins</strong></td>
<td>Average Kd ~1nM</td>
<td>Varies for different protein types</td>
<td>Median K&lt;sub&gt;d&lt;/sub&gt; ~1nM</td>
</tr>
<tr>
<td><strong>Multiplexing capability</strong></td>
<td>Upper limit ~40</td>
<td>No upper limit seen to date</td>
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</tr>
<tr>
<td><strong>Manufacturing consistency</strong></td>
<td>Produced in animals and recombinant methods Batch-to-batch variability</td>
<td>Chemically synthesized Minimal variability</td>
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</tr>
<tr>
<td><strong>Temperature and pH stability</strong></td>
<td>Requires cold storage Difficult to renature</td>
<td>Stable at ambient temp. Easily renatured</td>
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</tr>
<tr>
<td><strong>Diversity</strong></td>
<td>$10^{10}$ (based upon number of possible VDJ combinations)</td>
<td>$10^{13}$-$10^{15}$ (per library)</td>
<td>$10^{15}$ (per library)</td>
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