SOMAscan at the University of Houston

Somalogic's SOMAscan Assay allows for the simultaneous detection of 1,300 human proteins using SOMAmer (Slow Off-rate Modified Aptamer) protein-binding reagents. The assay is capable of detecting proteins in µM to fM concentrations by using dilutions of highly specific SOMAmers that bind to their cognate proteins. Bound SOMAmers are then quantified using a custom DNA microarray.

The MohanLab has been trained and certified as a Somalogic SOMAscan deployment site that is able to run human serum/plasma, cell lysate, and tissue homogenate. We are also able to run human urine and CSF as well as non-human plasma and serum. Sample volumes requested for each assay type is listed in Table 1 and recommended sample handling and processing procedures can be found on page 3.

Table 1. Human Matrices and Sample Size Requirements

Sample Type	Volume Sample Requested*
Serum	150 µL
CSF	150 µL
Urine	100 µL
EDTA Plasma	150 µL
Citrate Plasma (Liquid)	150 µL
Cell Lysates (CL)	75 µL @ 0.2 mg/mL
Cell Conditioned Media (CCM)	100 µL
Tissue Homogenate	75 µL @ 0.2 mg/mL

*Lower volumes may be feasible when availability is limited, enquire for more information.

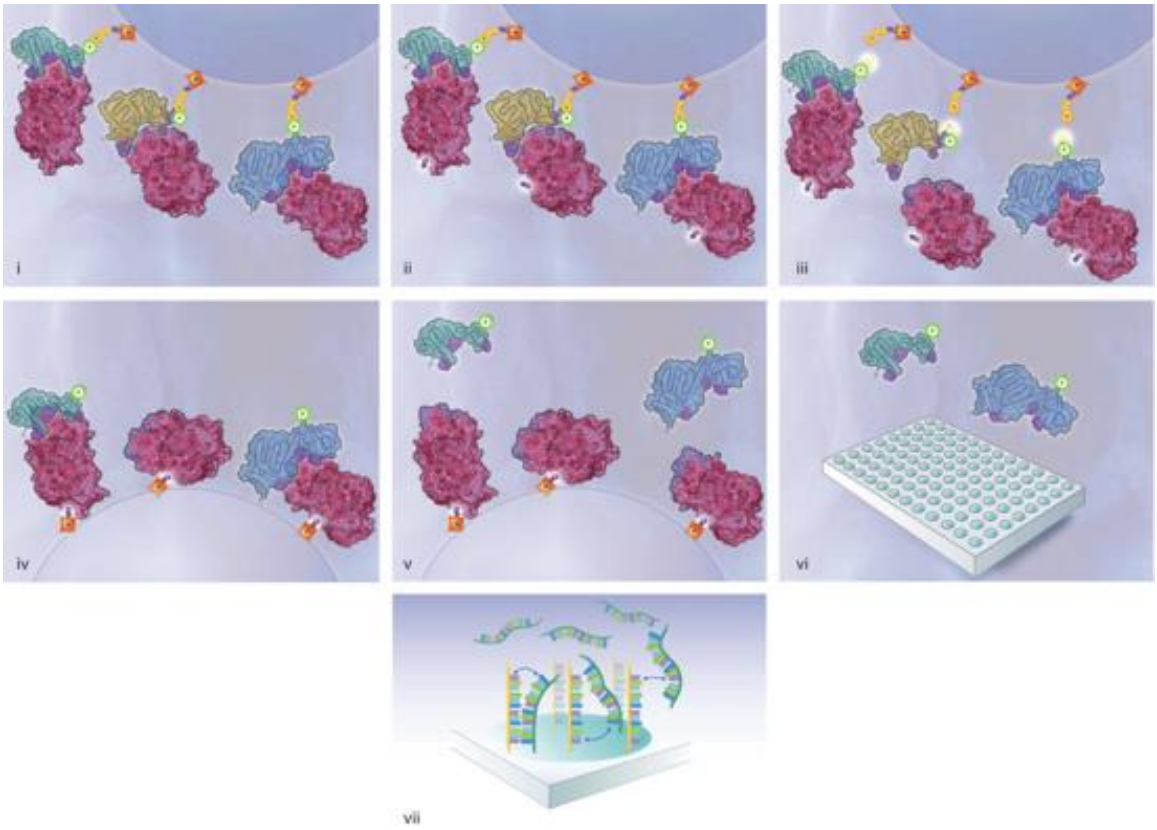
Each run assays **24 samples**. If you are interested in running more or less samples, please contact us for further accommodation. **Client pricing is USD 950 per sample** and discounted pricing is offered to **collaborators for USD 800 per sample** (and the latter requires prior approval).

After sample arrival at UH, assay processing and data processing will take up to **2 weeks.** The client will then receive the raw data files from the microarray analysis as well as files with recommended calibration by Somalogic Inc.

For more information, see Somalogic's website or a recent review article on this platform.

A list of proteins interrogated in the 1.3k scan can be found at http://mohanlab.bme.uh.edu/wp-content/uploads/2015/12/SSM-045-REV-1-SOMAscan-Assay-1.3k-Content-1.pdf.
Selected SOMAmers are also available for purchase through Somalogic's website: http://estore.somalogic.com/Default.aspx.

Highlights of the SOMAscan assay



(i) SOMAmer reagents attached to streptavidin beads bind to proteins in the sample mix
(ii) The proteins that are bound to their specific SOMAmer reagents are then biotinylated
(iii) The SOMAmer-protein complexes are released by photocleaving the linker, and non-specific SOMAmer-protein complexes are separated (center pair)
(iv) Biotinylated proteins are bound to a second streptavidin bead
(v) Bound SOMAmer reagents are removed from their protein targets
(vi) SOMAmer reagents are collected and denatured
(vii). SOMAmer reagents are measured using standard DNA analysis techniques like microarrays
More information can be found in the SOMAscan Assay Technical White Paper.

SOMAscan Assay: Recommended Sample Handling and Processing

Blood, Plasma, and Serum

The following procedures are recommendations for the collection and preparation of samples for assay on SOMAscan. Alternative methods that prevent protein denaturation can be used, but please consult with your SomaLogic representative to discuss the details prior to implementation.

Recommended blood sample collection protocol

Serum, EDTA, Heparin, and Citrate plasma are accepted.

- Check the expiration date on all of the tubes. If expired, replace with new ones.
- Perform the venipuncture per institutional guidelines.
- If more than one sample type is collected, follow the collection order according to tube manufacturer's guidelines.

General blood sample processing requirements

Proper processing of the collected samples is critical. Many tubes have a minimum and maximum fill line and these **requirements should be followed** and no additional additives should be added to the samples. It is particularly important that time constraints are observed and that samples are not left at room temperature longer than necessary. **Samples should be processed and frozen at -80 °C within 2 hours of collection.**

Note: Hemolyzed samples (pink to red in color) can confound true biomarker discovery. If available, send non-hemolyzed samples for your SOMAscan study. If only hemolyzed samples are available, please contact your SomaLogic representative.

Plasma processing

- Centrifuge plasma tubes (Citrate, Heparin or EDTA tubes) at room temperature. If within tube manufacture's specifications, spin at 2200 x g (***not* RPM**) for 15 minutes (this speed has been chosen to attempt to remove all cellular contents and platelets from samples). Observe separation of blood cells and plasma, with plasma layer on top.

- Draw off only the plasma layer. Take care not to disturb buffy coat when aliquoting by leaving some plasma behind and avoiding the cell layer. Aliquot into appropriately labeled tubes.

- Aliquot samples immediately and then place aliquoted samples in a -80 °C freezer. *Note: Plasma samples do not need to clot, and should be centrifuged immediately after collection.*

Serum processing

- Allow serum to clot for 60 minutes at room temperature prior to centrifugation.

- Centrifuge serum tubes. If within tube manufacture's specifications, spin at 2200 x g (***not* RPM**) for 15 minutes (this speed has been chosen to attempt to remove all cellular contents and platelets from samples). Observe separation of blood cells and serum, with serum layer on top.

- Draw off only the serum and aliquot into appropriately labeled tubes.

- Aliquot samples immediately and then place aliquoted samples in a -80 °C freezer.

Other Sample Types

Note: Your institutional procedure to harvest samples from patients should always be followed. The following are examples that have proven successful. The examples below do not preclude other methods, but please do contact your SomaLogic representative to discuss if your protocol deviates from what is described below.

Human cerebrospinal fluid (CSF) collection

The following protocol was provided by a collaborator.

- Perform Lumbar puncture (LP) in the morning after fasting since midnight to limit potential circadian fluctuation in CSF protein concentrations.
- Infiltrate the L3-4 or L4-5 interspace with 1% lidocaine using 25g needles for both superficial and deep local anesthesia.
- Perform LP with a 24g Sprotte bullet-tip atraumatic spinal needle using a 20g spinal introducer.
- Lumbar puncture should be performed with the patient in either the lateral decubitus or sitting position, according to the personal preference of the physician.
- CSF should be withdrawn using 5-mL sterile polypropylene syringes.
- The 15th to 25th mL of CSF collected should be retained for sample analysis.
- The sample should be aliquoted into 0.5 mL samples and the tubes should be labeled appropriately.
- Store aliquoted samples at -80 °C.

Urine

The protocol below have been used for samples assayed on SOMAscan and generally with good success.

- Collect neat urine sample.
- Clarify the urine by centrifugation at 14,000 x g for 5 minutes, prior to freezing, and collect the clarified supernatant.
- Store aliquots at -80 °C.

Cell culture lysate

Note: It is important that cells are harvested in aqueous solution under non-denaturing conditions. Whenever possible, cell lysates are preferred over cell supernatants containing serum. Serum can be washed away prior to lysing; the lysates can be normalized to total protein prior to running in the SOMAscan assay. The following protocol has been tested by SomaLogic and can be used for adherent cells and cell suspensions including lymphocytes.

- Collect samples using M-PER Mammalian Protein Extraction Reagent (Thermo Scientific) following manufacturer instructions.

- Sufficient material can usually be obtained from a cell monolayer, 80-100% confluent, in a single well of a six-well plate, harvested with 300 µL lysis buffer. (A rough guideline is ~133,000 cells, depending on cell type).

- To harvest cell lysate:

  -Wash cells three (3) times with Dulbecco's Phosphate Buffered Saline (DPBS) prior to lysing.

  -Add Halt protease inhibitor cocktail (Pierce Part# 78430) to the lysis buffer to inhibit protease activity, per kit instructions.

  -Add lysis buffer to the cells followed by appropriate lysation procedure.

  -Centrifuge lysed cells at 14,000 x g for 5 minutes, and collect the supernatant (clarified lysate).

- Quantify total protein amount using Micro BCA Protein Assay Kit (Thermo Scientific), or similar protein quantification method.

- Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS. Note: Submitted samples will be **assayed as-is**, so it is critical that samples are submitted at 200 µg/mL.

Cell conditioned media (Cell culture supernatants)

Note: The presence of serum (fetal bovine serum, bovine serum, horse serum, etc.) in conditioned cell media samples (i.e. cell culture supernatants) may impact the detection of small changes in proteins that are close to background levels. If possible, low or no serum is advised. If you are not sure of the effect of serum on the biology of interest and want to explore the smaller biological changes within your system, prepare samples ± serum or reduce the serum from 10% to 0.15% for the experiment.

SomaLogic has tested the assay performance of RPMI 1640 and DMEM (high glucose)

medias with phenol red, and penicillin and streptomycin**.**

- Serum can contribute proteins that cause signals in the assay. For studies with cells cultured in 1-10% fetal bovine serum, consider including proper control samples (media controls, untreated cells and/or vehicle-treated cells) depending on the scientific question to be addressed.

- Keep the media volume to a minimum in order to increase protein concentration, and strive to have cell density at 75% surface area or greater. Sufficient material can usually be obtained from 1 mL media removed from 80-100% confluent cell monolayer from a single well of a six-well plate.

- Time points of less than 24 hours may be too early to show a differential signal, consider reducing the volume of media used for these types of experiments.

- Clarify cell supernatant by centrifugation at 14,000 x g for 5 minutes, prior to freezing, and collect the clarified supernatant.

- The minimum volume required of clarified supernatant is 100 µL.

- Store samples in a -80 °C freezer.

Tissue or xenograft tumor homogenates

If you have a tissue that you would like to profile in the SOMAscan assay please enquire as to its feasibility.
Note: It is important that tissues are harvested in aqueous solution under non- denaturing conditions. Formalin-fixed tissues, or other denatured tissues, cannot be run in the SOMAscan assay. The protocols below are suggested protocols. Other protocols can be used provided they are non- denaturing. A brief consultation with SomaLogic is suggested prior to preparing the samples to evaluate protocol compatibility with SOMAscan. Also refer to: Alhamdani, M.S.S., et al. Journal of Proteome Research 2010, 9, 963-71.

Cryostat procedure

The following protocol was provided by a collaborator.

- Snap freeze tissue in frozen embedding medium within 5-10 minutes of excision.

- Keeping samples constantly frozen, cut five sections 10-micron thick, trim excess embedding medium from around tissue, and place tissue sections into a frozen sterile tube.

- Use T-Per tissue protein extraction agent (Thermo Scientific) per manufacturer's recommendation. Add 200 µL of buffer plus Halt protease inhibitor cocktail

(Pierce Part# 78430) per 10 mg of tissue.

- Homogenize in tube on ice with rotary pestle for 30 seconds, until no tissue fragments are visible.
- Centrifuge at >14,000 x g for 10 minutes while at 4 °C
- Filter supernatant through a 0.2 micron filter into a sterile tube or plate while on ice. (Millipore Multiscreen GV filter plate, 0.22 µm, sterile, Part # MSGV2210 or similar).
- Quantify total protein amount using Micro BCA Protein Assay Kit (Thermo Scientific), or other similar protein quantification method.
- Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS. Note: Submitted samples will be **assayed as-is**, so it is critical that samples are submitted at 200 µg/mL.
- Store aliquots at -80 °C.

Liquid nitrogen procedure

- Snap freeze (at least 5 mg) excised tissue in liquid nitrogen within 5-10 minutes of excision.
- Pulverize frozen tissue (using a freezer mill or similar) while maintaining low temperature using liquid nitrogen or dry ice.
- Use T-Per tissue protein extraction agent (Thermo Scientific) per manufacturer's recommendation. Add 200 uL extraction buffer plus Halt protease inhibitor cocktail (Pierce Part# 78430) per 10 mg of tissue.
- Homogenize in tube on ice with rotary pestle for 30 seconds, until no tissue fragments are visible.
- Centrifuge while cold at 14,000 x g for 10 minutes.
- Collect supernatant (keep on ice).
- Filter through a 0.2 micron filter into a sterile tube or plate.
- Quantify total protein amount using Micro BCA Protein Assay Kit (Thermo Scientific), or other similar protein quantification method.
- Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign

buffer such as PBS.

*Note: Submitted samples will be **assayed as-is**, so it is critical that samples are submitted at 200 µg/mL.*

Other samples including lymphocytes, bronchoalveolar lavage, exosomes, sputum, synovial fluid, tears, wound fluid, teeth, and nasal lavage have been assayed previously on SOMAscan. Please contact us for their detailed sample handling procedures.

Table 1. Human Matrices and Sample Size Requirements

Sample Type	Volume Sample Requested*
Serum	150 µL
CSF	150 µL
Urine	100 µL
EDTA Plasma	150 µL
Citrate Plasma (Liquid)	150 µL
Cell Lysates (CL)	75 µL @ 0.2 mg/mL
Cell Conditioned Media (CCM)	100 µL
Tissue Homogenate	75 µL @ 0.2 mg/mL
Everything not mentioned	Contact for required volume

*Lower volumes may be feasible when availability is limited, enquire for more information.

Non-human Samples in SOMAscan

SOMAmer reagents generated to human proteins have varying degrees of cross reactivity to non-human orthologs and therefore can be used to identify differential expression of some analytes in non-human samples. Please contact us for more information.