SOMAscan® Assay

Recommended Sample Handling and Processing

SomaLogic

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The following procedures are recommendations for the collection and preparation of samples for assay on SOMAscan at SomaLogic. Alternative methods that prevent protein denaturation can be used, but please consult with your SomaLogic representative to discuss the details prior to implementation. There may be sample types listed below that are not supported by kits to be run in SOMAscan Service Provider laboratories. If your samples will not be analyzed at SomaLogic, please consult with the SOMAscan Service Provider at your Institution.

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.

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.

Bronchoalveolar lavage (BAL)

Note: It is important to harvest in aqueous solution under non-denaturing conditions.

- Harvest BAL.
- Quantify total protein amount using Micro BCA Protein Assay Kit (Thermo Scientific), or similar protein quantification method.
- Normalize all samples to 90 µL at 200 µg/mL total protein concentration.
  Note: Submitted samples will be **assayed as-is**, so it is critical that samples are submitted at 200 µg/mL using a benign buffer such as PBS.
- Store BAL 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 Sample Types

The following matrices have been assayed less than 10 times in SOMAscan. SomaLogic has not determined the optimal sample processing protocol. The protocols below are those that have been used for samples assayed on SOMAscan and generally with good success.

Exosomes

Note: It is important that exosomes are harvested in aqueous solution under non-denaturing conditions.

- Isolate exosomes from the matrix of interest.
- Add sufficient volume of lysis buffer to the exosome pellet (e.g. 120 mM NaCl, 5 mM KCl, 5 mM MgCl2, 40 mM HEPES pH 7.5, 0.05% Tween20, 1% NP40 (v/v), 0.5% sodium deoxycholate (w/v)).
- Incubate samples for 15 minutes at 37 °C with mild agitation (rotation).
- Centrifuge for 5 minutes at 14,000 x g.
- Normalize all samples to 120 µl at 400 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 400 µg/mL.
- Store aliquots at -80 °C.

Sputum

Note: It is important that sputum is processed in aqueous solution under non-denaturing conditions.

- Samples collected by the Hargreave method have been assayed on SOMAscan. Djukanovic R., et al. European Respiratory Journal 2002, 20: Supplement 37
- Store aliquots at -80 °C.

Synovial fluid

Note: It is important that synovial fluid is processed in aqueous solution under non-denaturing conditions to pass through a 0.45 µm filter under modest vacuum.

- Samples processed by hyaluronidase digestion and bead ruptors have been assayed on SOMAscan.
- Store aliquots at -80 °C.

Tears

- SomaLogic has limited experience with tears. All interests require consultation and discussion. Store aliquots at -80 °C.

Wound fluid

- SomaLogic has limited experience with wound fluid. All interests require consultation

and discussion. Store aliquots at -80 °C.

Urine

- 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.

Teeth

SomaLogic has limited experience with teeth. All interests require consultation and discussion. Previous samples were prepared by the following:

- Pulverize teeth to create powder.
- Decalcify hard tissue powder with 0.5 M EDTA (pH 6.4) for 24h at 4 °C.
- Rinse the hard tissue powder thoroughly with deionized water.
- Re-suspend the tissue powder in extraction buffer consisting of 50 mM Tris-HCl, 5 mM $CaCl_2$, 0.9% NaCl, and 0.2% Tritin X-100 at 7.5 pH containing Halt protease inhibitor. The mixture is incubated for 24 h at 4 °C.
- Centrifuge sample at 14,000 rpm for 10 minutes.
- Collect supernatant.
- Quantify total protein amount using Micro BCA Protein Assay Kit (Thermo Scientific), or other 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 at -80 °C.

Nasal lavage

Note: It is important that nasal lavage is processed in aqueous solution under non-denaturing conditions.

The following is a commonly used Nasal lavage protocol:

- Collect nasal wash samples in normal unbuffered saline, 0.85% NaCl.
- The procedure is repeated twice on each naris for a total instilled saline volume of 3 mLs.
- Centrifuge at 450 x g for 10 minutes at 4 °C to remove cells.
- Recover supernatant and store at -80 °C

Sample Volume Requirements

Sample Volume Requirements

Table 1. Human Matrices and Sample Size Requirements

Species	Sample Type	Volume Sample Requested*	Tier
Human	EDTA Plasma	150 μL	1. Qualified: For intended purpose in biomarker discovery. Performance in SOMAscan extensively evaluated with assay parameters developed for matrix-specific optimization
	Serum	150 μL	
	CSF	90 μL	
	Heparin Plasma (Dry/Liquid)	150/160 μL	
	Cell Lysates (CL)	75 μL @ 0.2 mg/mL	
	Cell Conditioned Media (CCM)	100 μL	
	Citrate Plasma (Liquid)	150 μL	2. Developed: Performance in SOMAscan using existing parameters has been characterized
	Lymphocytes[A]	75 μL @ 0.2 mg/mL	
	Bronchoalveolar Lavage (BAL)[A]	90 μL @ 0.2 mg/mL	3. Research: Performance in SOMAscan using existing parameters has been minimally or not characterized. These matrices are run as standalone experiments requiring internal control
	Exosomes[A]	120 μL @ 0.4 mg/mL	
	Synovial Fluid[B]	250 μL	
	Tears in buffer	50 μL	
	Nasal Lavage	160 μL	
	Wound Fluid[B]	100 μL	
	Urine[B]	100 μL	
	Sputum[B]	120 μL	
	Teeth	75 μL @ 0.2 mg/mL	
	Tissue Homogenate	75 μL @ 0.2 mg/mL	
	Everything not mentioned	Contact SomaLogic for required volume	4. "Not tested": Matrix not tested on SOMAscan

[A]This sample type requires a total protein quantification assay prior to sample submission.

[B]Matrices that require additional pre-assay sample preparations at SomaLogic.

*Lower volumes may be feasible when availability is limited, enquire with SomaLogic.

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 refer to the *Non-human samples in the SOMAscan assay*

Technical Note, SSM-019, for more information. SSM-019 can be found on the SomaLogic website.

Table 2. Non-human species currently evaluated matrices and sample volumes required

Species	Sample Type	Volume Sample Requested*	Tier (As defined in Table 1)
Monkey	Plasma	130 µL	3. Research
	Serum	130 µL	
	CSF	150 µL	
Rat	EDTA Plasma	50 µL	3. Research
	Citrate Plasma	50 µL	
	Serum	50 µL	
	CSF	150 µL	
	BAL[A]	85 µL @ 0.25 mg/mL	
Mouse	EDTA Plasma	70 µL	3. Research
	Serum	70 µL	
	BAL[A]	85 µL @ 0.25 mg/mL	
Dog	EDTA Plasma	90 µL	2. Developed
	Serum	90 µL	3. Research
	Synovial fluid[B]	250 µL	
	Urine	80 µL	
Cat	EDTA Plasma	90 µL	3. Research
	Serum	90 µL	

[A]This sample type requires a total protein quantification assay prior to sample submission.
[B] Matrices that require additional pre-assay sample preparations.
**Lower volumes may be feasible when availability is limited, enquire with SomaLogic.*

New Matrices

We are continually investigating new sample types and are open to discussing additional sample types. If a matrix you would like to use in the SOMAscan assay is not on the list above, please enquire about the feasibility of evaluating it. New matrices are evaluated depending on resource availability and the size of the subsequent study. Any information regarding your matrix of interest that you can provide will help in the new matrix evaluation process.