SomaScan® Assay: Recommendations for Sample Handling and Processing

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The following procedures are recommendations for the collection and preparation of samples for Research Use Only assays on the SomaScan Platform. Other methods that prevent protein denaturation may be used, but please consult with your SomaLogic representative to discuss the details prior to implementation of an alternative collection protocol.

Updated from documents D0004350 "*The SomaScan Assay: Recommended Sample Handling and Processing for Core Sample Types*" and D0004351 "*The SomaScan Assay: Recommended Sample Handling and Processing for Non-Core Sample Types*"

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Table of Contents

1. Introduction

The SomaScan Assay can be used to analyze a wide variety of biological matrices and sample types and SomaLogic has developed multiple SomaScan Assay protocols to enable their analysis. This document addresses SomaLogic's approach to matrices, principles of the technology, and recommendations for the collection and preparation of samples for Research Use Only on the SomaScan Platform. Here you will find:

- A list of common specimen types which users have studied using the SomaScan Assay and associated volume requirements (shown in Table 1).
- Details about tube requirements for sample submission (refer to Section 3.1 or reach out to your SomaLogic representative)
- Guidelines for sample processing and handling (found in Sections 6 and 7).

Please contact your SomaLogic representative or techsupport@somalogic.com for additional guidance regarding specimen types in this table and those that are not listed.

Customers have two volume options when sending samples:

- one larger aliquot volume in a tube such as VWR microcentrifuge tube, or
- two smaller, assay-ready aliquots in the assay-ready matrix[1] tubes (notated in parenthesis in Table 1).

Table 1: Sample Types and Volume Requirements

Matrix	Required Sample Volume
Human plasma[3] (EDTA, citrate, CTAD, heparin; all collection tube types[2]) Non-human primate plasma Mouse, dog, cat, pig, marmot plasma	130 µL (two 55 µL aliquots, provided in **Matrix**[1] tubes)
Human serum (all collection tube types) Non-human primate serum Mouse, dog, cat, pig, marmot serum	130 µL (two 55 µL aliquots, provided in **Matrix**[1] tubes)
Human urine	550 µL (two 260 µL aliquots, provided in **Matrix**[1] tubes)
Human CSF Non-human CSF	SomaScan 7K Assay: 100 µL (two 40 µL aliquots, provided in **Matrix**[1] tubes)

Matrix	Required Sample Volume
	SomaScan 11K Assay: 75 µL (two 30 µL aliquots, provided in **Matrix**[1] tubes)
Human bone marrow aspirate Human synovial fluid (pre-treated)	130 µL (two 55 µL aliquots, provided in **Matrix**[1] tubes)
Cell lysate Tissue homogenate BAL Lymphocytes Amniotic fluid Nuclear extract Exosomes Wound Fluid Stool*	75 µL (two 30 µL aliquots, provided in **Matrix**[1] tubes) * *extracted stool samples must be received in two 30* µL *aliquots in* **Matrix**[1] *tubes*
Human aqueous humor Cell culture conditioned media Sputum Nasal lavage Vitreous humor	100 µL (two 40 µL aliquots, provided in **Matrix**[1] tubes)
Rat plasma Rat serum Saliva Blister Fluid Bovine plasma, milk Sheep plasma	50 µL (two 15 µL aliquots, provided in **Matrix**[1] tubes)

1. Thermo Scientific™ Matrix™ tubes, 0.5 mL screw top, 2D-barcoded (Thermo Scientific™ Catalog number: 3744. Alternatively, tubes 3745 with caps 4477).

2. Inform your SomaLogic representative if plasma samples were collected in cell-free DNA BCT tubes. Volume requirements are one 230 µL aliquot or two 115 µL aliquots if provided in Matrix[1] tubes.

3. Notify your SomaLogic representative if samples were provided by patients with Systemic Lupus Erythematosus (SLE). Volume required for submission is 75 µL or two 30 µL aliquots in our preferred Matrix[1] tubes.

For more detailed information regarding sample submission, please refer to Section 3.1.

2. Principle of the Assay

The SomaScan Assay measures thousands of unique human protein analytes in small volumes of biological samples in a high throughput fashion. The SomaScan Platform is enabled by the generation of protein-capture reagents called SOMAmer® (Slow Off-rate Modified Aptamer) reagents. The SomaScan Platform measures native proteins in complex matrices by transforming the available binding epitope on individual proteins into a corresponding SOMAmer reagent concentration, which is then quantified by hybridization to microarrays.

The SOMAmer reagents are supplied pre-bound on a magnetic bead resin in 96-well SOMAmer-bead plates. The neat biological samples and controls are diluted in a matrix-appropriate diluent. The SomaScan Assay plasma and serum protocols utilize three dilutions for every sample to accommodate the large concentration differences between proteins. Each sample dilution is applied to the corresponding bead plate containing subsets of SOMAmer reagents. Matrices such as urine, CSF, cell lysates, etc. are assayed with one SOMAmer-bead plate containing all SOMAmer reagents for the Single Dilution SomaScan Assay.

The diluted samples are pipetted into the appropriate SOMAmer-bead plate, where the proteins in the sample bind to the SOMAmer reagent.

After this binding step, any unbound substances (proteins, matrix constituents, diluents) are washed away and a biotinylation reagent is added to label the proteins which are bound. Subsequent washes remove any excess biotinylation reagent.

Next, the samples are exposed to UV light which releases all SOMAmer reagents and the bound proteins from the bead resin. The solution is transferred to a new 96-well plate containing a set of streptavidin coated magnetic beads.

The biotinylated protein-SOMAmer complex binds to the magnetic beads and a series of wash steps are completed to remove any SOMAmer reagents that did not bind to a protein.

Next, an elution buffer is used to disassociate the bound SOMAmer reagents from their associated protein. The resulting eluate is collected and transferred to a new 96-well plate. The eluted SOMAmer reagents are added to microarrays where they bind to complementary probes on the array during a hybridization reaction.

Following microarray washing, the microarray slides are processed in an Agilent microarray scanner system where a laser excites the fluorophore intrinsic to each SOMAmer reagent. The fluorescence intensity is proportional to the available epitopes of the corresponding proteins in the original sample.

For more information, refer to "*SomaScan® Assay v4.1 Technical Note*" (SL00000572) and "*SomaScan® 11K Assay v5.0 Technical Note*" (SL00000919).

3. General Sample and Study Recommendations

Sample volume requirements are dependent upon the specimen type. Please see the following sections in the appendices relevant to your matrix or refer to **Table 1**. For samples where the submission requirements cannot be met or if you have any questions or concerns regarding sample logistics, such as volume needs, tube types, import and export permits, known infectious materials, or successful couriers, please reach out to your SomaLogic Project Manager or email techsupport@somalogic.com.

3.1. Tube Recommendations for Sample Submission

Sample handling post-receipt will be minimized by providing aliquots in tubes that are compatible with SomaLogic's tracking and automation systems. To streamline your assay in the assay queue we recommend that customers send the required aliquot volumes in **0.5 mL screw top, 2D-barcoded, Thermo Scientific™ Matrix™ tubes** (Thermo Scientific™ Catalog number: 3744. Alternatively, tubes 3745 with caps 4477). For instance, submit two matrix tubes with exactly 55 µL plasma in each tube. Please do not send pre-aliquoted samples in other tube types.

If any other tube is being used, please provide samples as one large aliquot (refer to Table 1) in a single tube such as a VWR Microcentrifuge tube (VWR Catalog number: 10025-748; 20170-038). Two aliquots will be removed from the parent sample after receipt and stored at -80ºC until the SomaScan Assay.

Our Project Management team will coordinate any residual sample disposal. Remaining volume can be returned, at shipping costs, or destroyed by a third-party vendor.

Please note that sample submission and shipping requirements may differ between SomaLogic's Boulder headquarters and external Authorized Sites. Please contact the Authorized Site to which you plan to send your samples for further information.

3.2. Additional Notes

Customers should plan to submit all samples necessary for their analysis (e.g. healthy and diseased, untreated and treated, control population, etc.) and are responsible for the quality of the samples sent to SomaLogic. SomaLogic does not re-run individual samples that are flagged for exceeding typical ranges for normalization scale factors. Please see the “Filtering, Interpretation and Considerations for Flagged Samples in the SomaScan® Assay" technical note (D0006601) for more information. SomaLogic will re-analyze whole plates that do not meet the acceptance criteria based on SomaLogic internal control samples.

Consistency in sample collection and processing is critical to reduce bias that can confound biological interpretation. Proteomic comparisons ideally should be made across one sample type and RFU signals should not be directly compared across different matrices. For example, it is not advisable for a study to consist of both EDTA-plasma samples and heparin-plasma samples. Likewise, it is not advisable to compare signals across cell lysates and cell supernatants. Each specimen type will be considered an independent project/study and customers will receive one data package per study. One should consider assessing biological trends in one matrix to compare with biological trends in another matrix.

4. Standard Sample Types

SomaLogic routinely runs a variety of specimen types- human plasma, human serum, human urine and human CSF are considered standard matrices. Sample recommendations for collection, processing and storage can be found in Section 6.

To remove systematic biases and ensure assays perform accordingly, each 96-well sample plate is run with **SomaLogic internal controls** (5 calibrators, 3 QC samples, 3 No Protein samples). This allows for various layers of normalization to remove systematic biases such as sample-by-sample, plate-to-plate, and SOMAmer-by-SOMAmer (refer to SL00000048 and SL00000752). Quality control (QC) sample accuracy is used to assess plate-level performance and must meet certain acceptance criteria before data is released to the customer.

For the SomaScan 7K Assay, human EDTA-plasma, human serum, human urine, and human CSF have undergone **method validation of performance metrics** utilizing this control strategy and ANML normalization discussed below. These metrics can include reproducibility, precision, sensitivity, dynamic range, signal-to-noise, population F-statistics and more. Information about performance metrics on a SOMAmer-by-SOMAmer reagent basis for EDTA-plasma and serum can be found at https://menu.somalogic.com/.

Currently, the SomaScan 11K Assay validated sample types are human EDTA-plasma, human serum, and human CSF. See "*SomaScan® Assay Technical Note*" (SL00000572) for more information about the SomaScan Platform.

Validated matrices also utilize an exclusive data standardization method called adaptive normalization by maximum likelihood (ANML). ANML is a unique SomaLogic normalization capability that mitigates sample-specific bias by centering total signal to a population reference, therefore strengthening reproducibility and confidence in detecting biological differences (see "*SomaScan® v4 Data Standardization and File Specification Technical Note*" SL00000048 and "*Calibration and Normalization Technical Note for Single Dilution Kits"* SL00000752). **Because of this, repeated or bridging samples for combining data sets across extended studies are not necessary for these validated specimen types.**

Other plasma anticoagulants including, but not limited to, citrate and heparin are also acceptable and commonly assayed, but have not been characterized for the same performance metrics as EDTA-plasma. Directly comparing samples across different matrices (such as EDTA-plasma and heparin plasma) is not advised since proteomic profiles can vary simply based on differences in tube anticoagulant.

Normalization to a reference standardizes the overall signal for every sample and is appropriate for experiments that generally match conditions of the reference population. Plasma samples collected with anticoagulants other than EDTA and citrate will require an alternative normalization process used for non-standard matrices (see section 5 below). For studies with inconsistent protein amounts (e.g., Duchenne Muscular Dystrophy or leukemic blood), or studies where subjects differ significantly from this reference, such as,

- Pregnancy
- Pediatric
- Post-traumatic injury

- Perishing (near death, sepsis, organ failure)
- Peri-operative (propofol anesthesia)
- Systemic Lupus Erythematosus (SLE)(see "Additional Notes" below)
- Large effects from pre-analytical variation

an elevated number of samples may be flagged to signify outliers and an alternative normalization process may also be more appropriate. Please see the "*Filtering, Interpretation and Considerations for Flagged Samples in the SomaScan® Assay*" technical note (D0006601) for more information or notify your SomaLogic representative. For projects expected to span across batches over time with these types of conditions, re-running a subset of samples should be considered if users wish to utilize their own normalization methods for combining data.

4.1. Additional Notes

- Strive for consistency in sample collection, processing, and storage to minimize confounding factors due to pre-analytical variation (PAV). See SomaLogic's Tech Note "*Pre-analytical variation assessment for more reliable results*" (SL00000713) and "*Assessing pre-analytical variation*" white paper (SL00000716) or contact techsupport@somalogic.com to learn more about SomaLogic tools that can help decipher impacts from PAV in human EDTA-plasma and serum.
- Due to their physiological compositions, not all analytes on the SomaScan menu are expected to signal above background for human urine and human CSF. Of the SomaScan 7K Assay menu, 2893 Human Proteins in urine and 4156 Human Protein in CSF were in the S2 signaling group, meaning the protein signal to noise ratio was consistently greater than or equal to 2 and less than saturation from a set of normal samples. Refer to "*Calibration and Normalization Technical Note for Single Dilution Kits"* SL00000752 and "*Urine Measurements on the SomaScan® v4.1 Assay"* SL00000613 for more information.
- ***Notify your SomaLogic representative if samples were provided by patients with Systemic Lupus Erythematosus (SLE).***
- ***Notify your SomaLogic representative if plasma samples were collected in STRECK cell-free DNA BCT tubes.***

5. Alternative Sample Types

Alternative matrices have been successfully run on the SomaScan Assay, however, please note that dilution groups, diluents, and control samples have not been optimized for non-human or other alternative sample types. Results will vary depending physiological ranges of proteins, matrix composition, sample prep methods, and specific biological and experimental conditions. SomaLogic has not validated alternative matrices for the metrics described in the previous section. Preliminary development has been done on some alternative matrices (notably those in Table 1) to the extent that SomaLogic has narrowed down the range of suitable run conditions such as pre-assay sample dilution or pre-assay concentration normalization.

SomaLogic includes internal controls on every 96-well assay plate and are run with alternative matrices. Precision estimates and assay performance are determined by an assessment of the internal calibrator control replicates. Methods used to mitigate sample-specific bias for alternative sample types utilize a normalization strategy where signals are centered around a median total signal from the study samples themselves, referred to as intra-study median signal normalization (*"Calibration and Normalization Technical Note for Single Dilution Kits"* SL00000752).

5.1. Non-Human Proteins

SOMAmer reagents are generated to human proteins and bind to a specific epitope on the protein target it was selected against. Conservation of epitopes allows varying degrees of cross reactivity to non-human orthologs and therefore the SomaScan Assay has been used to identify differential expression in non-human samples. In a small-scale study, about 200 SOMAmer reagents showed binding generally correlated with amino acid sequence similarity between human and mouse proteins. Direct cross-reactivity to non-human proteins has not been assessed in a larger context, however a larger scale study on the SomaScan 7K Assay measuring individual mouse, dog, rat and hamster plasma samples and pooled titrations showed 72% of content signaling in rat and more than 86% signaling in these other species. For more information, please refer to "*SomaScan™ Assay Signaling in Non-Human Plasma* " (SL00000795) or contact techsupport@somalogic.com.

5.2. Best Practices for Alternative Matrices

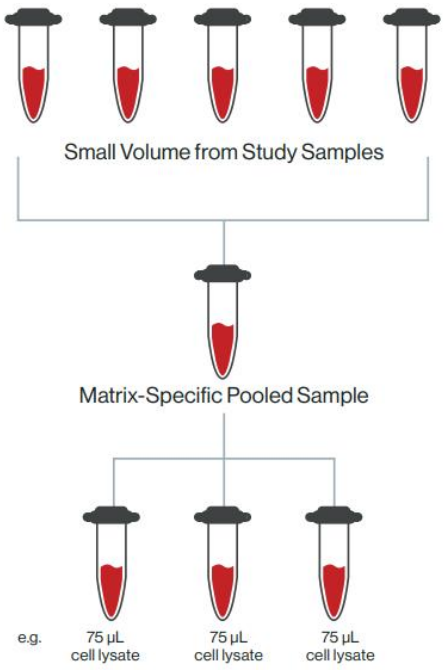
Your institutional procedure to harvest samples from patients should always be followed. The sample handling and processing recommendations in Section 7 are examples that have proven successful. The examples do not preclude other methods, but please reach out to your SomaLogic representative or techsupport@somalogic.com if your protocol deviates from what is described below.

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

It is important to plan ahead and consider any samples that are necessary to answer all specific study questions. In addition to making comparisons across different groupings of samples (e.g. healthy and diseased, untreated and treated, longitudinal studies, etc.) it can also be useful to understand things like assay technical variability, background, or study to study reproducibility of a unique matrix.

Provided below are common analyses and samples that customers may consider including in their study submission if they plan to assess these different components during data analysis.

- **Technical Replicates (3-5 samples)**: To assess the reproducibility of unique sample types and inter-run batch effects, it can be useful to include at least three to five matrix-specific technical replicates from an individual sample on each SomaScan Assay plate (85 or 90 samples are run per plate, depending on matrix). One option is to make a larger matrix-specific pooled sample by combining a small volume from each study sample into one tube. This prevents any one sample from being depleted and the pooled sample is representative of all study samples. Then thoroughly mix and pipette into multiple aliquots to include with your study submission (see right image).



Submit technical triplicates (at least) with your sample set

- **No Protein Replicates (3-5 samples)**: Including preparation-specific No Protein samples can allow you to assess the assay background of your unique matrix. Examples include extraction buffer, cell media, storage buffer, or other background controls. SomaLogic includes their own internal No Protein buffer samples on each plate. However, by providing matrix-specific No Protein samples, the background signal will be more reflective of your unique matrix.

- **Repeated Samples for Extended Studies (*number of samples- discretionary*)**: When samples are expected to span across multiple studies, repeating a subset may be useful and provide a more formal evaluation to assess batch quality and affects. In this context, a 'batch' refers to any technical factor that may introduce systematic artifacts into the data, potentially affecting its integrity or interpretation (e.g. plate, submission 'batch' or site). Should an artifact be identified, they may also be used to perform adjustments for its correction.

6. Handling and Processing Recommendations for Standard Samples

Please see Section 3 for General Sample and Study guidance and Section 4 for general information about standard sample types. The sample collection and storage conditions listed below are intended as general guidelines. Exhibit caution when using alternative methods not outlined in this document as impacts to data may be unknown. ***Regardless of the preparation method, customers should strive for consistency across all samples for collection, processing, and storage temperature to minimize confounding factors due to pre-analytical variation.*** For questions about the SomaLogic technology, sample prep or for any other scientific inquiries, please contact techsupport@somalogic.com.

For blood samples like plasma and serum, proper processing of the collected samples is critical.

- Check the expiration date on all tubes. If expired, replace with new ones.
- Perform the venipuncture and tube mixing per institutional guidelines.
- If more than one sample type is collected, follow the collection order according to tube manufacturer's guidelines.
- Many tubes have a minimum and maximum fill line, and these requirements should be followed with no additional additive added to the samples.
- We encourage sample processing time constraints be observed and that samples are not left at room temperature longer than necessary. Plasma and serum samples should be processed and frozen at -80°C within 2 hours of blood collection.

6.1. Plasma Processing

1. Perform all steps at room temperature. Do not chill the vacutainer prior to processing.
2. Centrifuge plasma tubes at room temperature. If within tube manufacture's specifications, spin at 2,200 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.
3. Draw off only the plasma layer. Take care not to disturb buffy coat when aspirating by leaving some plasma behind and avoiding the cell layer.

4. Aliquot the sample immediately into appropriately labeled tubes (130 µL total sample or two 55 µL aliquots in matrix[1] tubes).
5. Aliquots should be stored at -80°C until submission to SomaLogic or a SomaLogic Authorized Site. Storage effects at -20°C have not been evaluated for the SomaScan 7K and 11K Assays.

Note: Plasma samples do not need to clot and should be centrifuged immediately after collection.

6.2. Serum Processing

1. Perform all steps at room temperature. Do not chill the vacutainer prior to processing.
2. Allow serum to clot for 60-90 minutes at room temperature prior to centrifugation.
3. Centrifuge serum tubes at room temperature. If within tube manufacture's specifications, spin at 2,200 x g (not RPM) for 15 minutes (this speed has been chosen to attempt to remove all cellular contents and platelets from samples). Standard centrifugation of 1,300 x g for 10 minutes provides comparable results. Ensure separation of blood cells and serum is observed, with serum layer on top.
4. Draw off only the serum layer.
5. Aliquot sample within 30 min of centrifugation into appropriately labeled tubes (130 µL sample total or two 55 µL aliquots in matrix[1] tubes).
6. Aliquots should be stored at -80°C until submission to SomaLogic or a SomaLogic Authorized Site. Storage effects at -20°C have not been evaluated for the SomaScan 7K and 11K Assays.

6.3. Urine Processing

1. Collect neat urine midstream catch.
2. All study samples should be handled in the same way (e.g. centrifuged at a given speed and time or not at all).
3. The minimum volume required of urine is 550 µL (or two 260 µL aliquots in matrix[1] tubes).
4. Store aliquots at -80°C immediately.

6.4. Cerebrospinal Fluid (CSF) Collection

The following protocol was provided by a collaborator.

1. 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 25 gauge (G) needles for both superficial and deep local anesthesia.

2. Perform LP with a 24 G Sprotte® bullet-tip atraumatic spinal needle using a 20 G spinal introducer.
3. Lumbar puncture should be performed with the patient in either the lateral decubitus or sitting position, according to the personal preference of the physician.
4. CSF should be withdrawn using 5 mL sterile polypropylene syringes.
5. The 15th to 25th mL of CSF collected should be retained for sample analysis.
6. The sample should be labeled appropriately and have a minimum volume of:
    a. SomaScan 7K Assay: 100 µL (or two 40 µL aliquots in matrix[1] tubes)
    b. SomaScan 11K Assay: 75 µL (or two 30 µL aliquots in matrix[1] tubes)
7. Store samples at -80°C.

7. Handling and Processing Recommendations for Alternative Samples

Please see section 3 for General Sample and Study guidance and section 5 for additional guidance pertaining to alternative sample types.

Your institutional procedure to harvest samples from patients should always be followed. The sample handling and processing recommendations presented here are examples that have proven successful. The examples do not preclude other methods, but please contact your SomaLogic representative or techsupport@somalogic.com if your protocol deviates from what is described below.

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

7.1. Bronchoalveolar Lavage (BAL)

1. Harvest BAL.
2. Quantify total protein amount using Micro BCA™ Protein Assay Kit (Thermo Scientific™) or similar protein quantification method.
3. Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS (or two 30 µL aliquots in matrix[1] tubes).
4. Store samples at -80°C.

7.2. 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 homologous. 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.

For studies with cells cultured in serum, consider including proper control samples (media controls, untreated cells and/or vehicle-treated cells) depending on the scientific question to be addressed.

SomaLogic has tested the assay performance of RPMI 1640 (Gibco™) and DMEM (high glucose) (Gibco™) medias with phenol red, penicillin, and streptomycin.

1. Serum can contribute proteins that cause signals in the assay.

2. 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.
3. Time points shorter than 24 hours may be too early to show a differential signal; consider reducing the volume of media used for these types of experiments.
4. Clarify cell supernatant by centrifugation at 14,000 x g for 5 minutes and collect the clarified supernatant.
5. The minimum volume required of clarified supernatant is 100 µL (or two 40 µL aliquots in matrix[1] tubes).
6. Store samples at -80°C

7.3. 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.

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).

1. To harvest cell lysate:
    - Wash cells three (3) times with ice cold Phosphate Buffered Saline (PBS) prior to lysing.
2. Collect samples using M-PER™ Mammalian Protein Extraction Reagent (Thermo Scientific™) following manufacturer instructions.
    - Add Halt™ protease inhibitor cocktail (Thermo Scientific™) to the lysis buffer to inhibit protease activity, per kit instructions.
    - Add lysis buffer to the cells followed by appropriate lysis procedure.
    - Centrifuge lysed cells at 14,000 x g for 5 minutes and collect the supernatant (clarified lysate).
3. Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS (or two 30 µL aliquots in matrix[1] tubes).
4. Store samples at -80°C.

7.4. Exosomes

Note: It is important that exosomes are harvested in aqueous solution under non-denaturing conditions. ExoQuick has been found to be denaturing and should be avoided.

1. Isolate exosomes from the matrix of interest.
2. Add sufficient volume of lysis buffer to the exosome pellet (e.g., 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 40 mM HEPES pH 7.5, 0.05% Tween20, 1% NP40 (v/v), 0.5% sodium deoxycholate (w/v)).
3. Incubate samples for 15 minutes at 37°C with mild agitation (rotation).
4. Centrifuge for 5 minutes at 14,000 x g.
5. Quantify total protein amount using Micro BCA™ Protein Assay Kit (Thermo Scientific™) or similar protein quantification method.
6. Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS (or two 30 µL aliquots in matrix[1] tubes).
7. Store samples at -80°C.

7.5. 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:

1. Collect nasal wash samples in normal unbuffered saline, 0.85% NaCl.
2. The procedure is repeated twice on each naris for a total instilled saline volume of 3 mL.
3. Centrifuge at 450 x g for 10 minutes at 4°C to remove cells.
4. Recover supernatant.
5. The minimum volume required is 100 µL (or two 40 µL aliquots in matrix[1] tubes).
6. Store samples at -80°C.

7.6. Sputum

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

1. Samples collected by the Hargreave method have been assayed on the SomaScan platform. Djukanovic R., et al. European Respiratory Journal 2002, 20: Supplement 37.
2. The minimum volume required is 100 µL (or two 40 µL aliquots in matrix[1] tubes).
3. Store samples at -80°C.

7.7. Stool

Note: Stool samples must be processed and pre-aliquoted into the required matrix tubes: 0.5 mL screw top, 2D-barcoded, Thermo Scientific™ Matrix™ tubes (Thermo Scientific™ Catalog number: 3744. Alternatively, tubes 3745 with caps 4477). SomaLogic will return samples directly to the shipper if they arrive unprocessed.

It is important that stool is extracted in aqueous solution under non-denaturing conditions.

Protocols for stool samples that have been collected, processed and then assayed on the SomaScan platform can be found in the following publications:

- Li, H., Vanarsa, K., Zhang, T. et al. Comprehensive aptamer-based screen of 1317 proteins uncovers improved stool protein markers of colorectal cancer. J Gastroenterol 56, 659–672 (2021).
- Soomro, S., Venkateswaran, S., Vanarsa, K. et al. Predicting disease course in ulcerative colitis using stool proteins identified through an aptamer-based screen. Nat Commun 12, 3989 (2021).

1. Quantify total protein amount using Micro BCA™ Protein Assay Kit (Thermo Scientific™) or similar protein quantification method.
2. Normalize all samples to 200 µg/mL total protein concentration using a benign buffer such as PBS.
3. Samples must be aliquoted into two 30 µL aliquots using the required matrix[1] tubes mentioned above.
4. Store samples at -80°C.

7.8. Synovial Fluid

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

- Synovial fluid must be treated by the client to reduce viscosity (enzymatic or mechanical).
- Samples processed by hyaluronidase digestion and bead ruptors have been assayed on the SomaScan platform.
- The minimum volume required is 130 µL (or two 55 µL aliquots in matrix[1] tubes).
- Store samples at -80°C.

7.9. Tissue or Xenograft Tumor Homogenates

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. You may also refer to: Alhamdani, M.S.S., et al. *Journal of Proteome Research* 2010, 9, 963-71.

Cryostat Procedure

1. Snap freeze tissue in frozen embedding medium within 5-10 minutes of excision.
2. Keeping samples constantly frozen, cut five sections 10 µm thick, trim excess embedding medium from around tissue, and place tissue sections into a frozen sterile tube.
3. Use T-PER™ tissue protein extraction reagent (Thermo Scientific™) and Halt™ protease inhibitor cocktail (Thermo Scientific™) per manufacturer's recommendation. Generally, a 1:20 (w/v) ratio of tissue to T-PER reagent Is used, however a smaller volume may be used to yield a more concentrated protein sample.
4. Homogenize in tube on ice with rotary pestle for 30 seconds, until no tissue fragments are visible. Bead ruptor homogenizers can also be used and should be processed in the fridge to keep samples cool.
5. Centrifuge at 10,000 x g for 5 minutes while at 4°C.
6. Collect supernatant (keep on ice).
7. Optional: Filter supernatant through a 0.2 µm filter into a sterile tube or plate while on ice. (Millipore® Multiscreen® GV filter plate, 0.22 µm, sterile, Part # MSGV2210 or similar).
8. Quantify total protein amount using Micro BCA™ Protein Assay Kit (Thermo Scientific™) or other similar protein quantification method.
9. Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS (or two 30 µL aliquots in matrix[1] tubes).
10. Store samples at -80°C.

Liquid Nitrogen Procedure

1. Snap freeze (at least 5 mg) excised tissue in liquid nitrogen within 5-10 minutes of excision.
2. Pulverize frozen tissue (using a freezer mill or similar) while maintaining low temperature using liquid nitrogen or dry ice.
3. Use T-PER™ tissue protein extraction reagent (Thermo Scientific™) and Halt™ protease inhibitor cocktail (Thermo Scientific™) per manufacturer's recommendation. Generally, a 1:20 (w/v) ratio of tissue to T-PER reagent Is

used, however a smaller volume may be used to yield a more concentrated protein sample.

4. Homogenize in tube on ice with rotary pestle for 30 seconds, until no tissue fragments are visible. Bead ruptor homogenizers can also be used and should be processed in the fridge to keep samples cool.
5. Centrifuge at 10,000 x g for 5 minutes while at 4°C.
6. Collect supernatant (keep on ice).
7. Optional: Filter through a 0.2 µm filter into a sterile tube or plate.
8. Quantify total protein amount using Micro BCA™ Protein Assay Kit (Thermo Scientific™) or other similar protein quantification method.
9. Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS (or two 30 µL aliquots in matrix[1] tubes).
10. Store samples at -80°C.

7.10.Wound Fluid

1. Quantify total protein amount using Micro BCA™ Protein Assay Kit (Thermo Scientific™) or similar protein quantification method.
2. Normalize all samples to 75 µL at 200 µg/mL total protein concentration using a benign buffer such as PBS (or two 30 µL aliquots in matrix[1] tubes).
3. Store samples at -80°C.

For help and technical information, please submit your questions to techsupport@somalogic.com or call 1-844-SOMAmer.

8. Referenced Documents

Document ID	Document Title
D0006601	Filtering, Interpretation and Considerations for Flagged Samples in the SomaScan® Assay
SL00000048	SomaScan® v4 Data Standardization and File Specification Technical Note
SL00000572	SomaScan® Assay v4.1 Technical Note
SL00000919	SomaScan® 11K Assay v5.0 Technical Note
SL00000716	Assessing pre-analytical variation White Paper
SL00000713	Pre-analytical variation assessment for more reliable results
SL00000795	SomaScan™ Assay Signaling in Non-Human Plasma
SL00000752	Calibration and Normalization Technical Note for Single Dilution Kits