

# SomaScan® 7K Assay v4.1: Sample Preparation Urine Kit User Manual

September 2023

This user manual describes the processing steps for human biobank urine samples using the Single Dilution SomaScan Assay 7K Kit and is applicable to kit part number 900-00026.

#### Manufactured and distributed by:

SomaLogic Operating Co., Inc. 2945 Wilderness Place, Boulder, CO 80301

Phone: (303) 625-9000

Email: info@somalogic.com

D0004921 Rev1: 2023-09

### **Table of Contents**

1.	In	troduction	3
1	1.1	For Research Use Only	3
2.	Μ	laterial Provided & Storage Conditions	4
3.	0	ther Supplies Required, but Not Supplied in the SomaScan Assay 7K – Urine K	t.4
4.	S	ample Collection and Storage	4
5.	С	onsiderations for the Pre-processing and Protein Quantification	5
6. ~2		etup and Implementation of SomaScan 7K Sample Preparation: Urine (Durati	
(	6.1.	Consumable Preparation	5
(	6.2.	Tecan Fluent Startup and Platform Initialization	5
(	6.3.	Reagent and Sample Preparation	8
(	6.4.	SomaScan 7K Sample Preparation: Urine	10
(	6.5.	Binding Reaction	14
(	6.6.	Robot Cleanup	14
(	6.7.	Sample Plate Map	15
7. Pre		omaScan Assay 7K Kit - Single Dilution Assay Instructions: Auxiliary Reagent (Duration: ~10 min)	16
-	7.1.	Auxiliary Preparation	16
-	7.2.	Sample Documentation	17
8.	R	eference Documents	18
Αp	pe	ndix 1: Consumable List	19
Αp	pe	ndix 2: Instructions for Adjustment of RGA Gripper Fingers	19
Αp	ре	ndix 3: Components Associated with the Urine KitKit	. 20

#### 1. Introduction

This document describes the sample preparation procedure for the SomaScan Assay 7K Kit for Urine for the analysis of up to 85 urine samples using kit part number 900-00026. The completion of this protocol must be followed by the execution of the Single Dilution SomaScan Assay 7K protocol, as described in D0004923 (SomaScan Assay 7K Kit - Single Dilution Assay Instructions). Performing protein quantification using a micro BCA assay on the pH adjusted and buffer exchanged urine samples (D0005009) is required prior to completing this protocol.

The data from the micro BCA protein quantification will be used to dilute the samples twice if the calculated protein concentration is above 83.35  $\mu g/mL$ . The first pre-dilution is in Assay Buffer (AB) to normalize the total protein concentration to 83.35  $\mu g/mL$ . The second dilution is completed using a urine specific diluent to achieve a final total protein concentration of 75  $\mu g/mL$ . If the calculated protein concentration for a urine sample from a micro BCA assay is less than or equal to 83.35  $\mu g/mL$ , there will be one dilution step in urine specific diluent with a final concentration of 90% of the calculated value.

#### 1.1 For Research Use Only

The SomaScan Assay 7K, SomaScan Assay 7K kits, and SOMAmer® reagents may only be used for their intended purpose as described in, and in strict accordance with, the protocols and other materials accompanying them. The SomaScan Assay 7K, SomaScan Assay 7K kits, and SOMAmer reagents are developed, designed, intended, and sold to the institution for research purposes only and are not for use in diagnostic procedures. The SomaScan Assay 7K, SomaScan Assay 7K kits, and SOMAmer reagents are not to be used by the institution or any third party for human consumption, diagnostic, clinical or therapeutic applications, or be included or used in any drug intended for human, agricultural or cosmetic use. All care and attention should be exercised in the handling of the SomaScan Assay 7K, SomaScan Assay 7K kits, and SOMAmer reagents by following appropriate research lab practices.

Additional limitations of the procedure are described in document D0004919 SomaScan Assay 7K Kit - Overview and Introduction.

### 2. Material Provided & Storage Conditions

The SomaScan Assay 7K – Urine Kit (900-00026) is shipped at three conditions – one on dry ice, one on wet ice, and one at ambient temperature. Upon receipt of the SomaScan Assay 7K – Urine Kit, the components should be stored at the indicated temperatures:

Kit Component Name	Part Number	Storage Temperature
SomaScan Assay 7K, ambient	899-00042	Ambient
components	699-00042	(+10 to +30 °C)
Compactor Access 71/ 4C company and	899-00043	+4 °C
SomaScan Assay 7K, 4C components	899-00043	(+2 to +8 °C)
Access Buffer 1000 pol	CE1 0010E	+4 °C
Assay Buffer, 1000 mL	651-00125	(+2 to +8 °C)
SomaScan Assay 7K, -20C Single	000 00050	-20 °C
Dilution components	899-00050	(-10 to -30 °C)
SomaScan Assay 7K, -80C Urine	000 00050	-80 °C
components	899-00052	(-70 to -90 °C)

For a detailed list of the components provided in the SomaScan Assay 7K – Urine Kit , as well as all other types of kits available, please see document D0004919.

# 3. Other Supplies Required, but Not Supplied in the SomaScan Assay 7K – Urine Kit

Please refer to D0004924 SomaScan Assay 7K Kit - Single Dilution Assay Consumable List or **Appendix 1** at the end of this document for a complete list of consumables required to complete this protocol. This list also includes all consumables required to perform the subsequent Single Dilution SomaScan Assay 7K, as described in the manual D0004923.

**NOTE:** Do not proceed with this procedure unless all consumables have been obtained.

### 4. Sample Collection and Storage

For an example of the sample collection procedure for urine samples, see D0004350 SomaScan Assay: Recommended Sample Handling and Processing for Core Sample Types.

**NOTE:** Sample stability has not been evaluated.

# 5. Considerations for the Pre-processing and Protein Quantification

Urine sample must be pH adjusted, buffer exchanged, and have protein quantification analysis via a micro BCA assay prior to initiating this protocol. This pre-processing can occur any time prior to starting the urine sample preparation and SomaScan Assay 7K procedure. See the **Urine Pre-processing – User Manual D0005009** for instructions. The measured protein concentration by micro BCA assay is a required input for this sample preparation procedure.

# 6. Setup and Implementation of SomaScan 7K Sample Preparation: Urine (Duration: ~2 h)

#### 6.1. Consumable Preparation

- Obtain one Nunc round bottom plate and label as follows on the front and lefthand side with the notch facing towards the analyst and in the lower left-hand corner
  - o Dil 1 (D1)
- Obtain one 2 mL deep-well plate and label as follows on the front and lefthand side with the notch facing towards the analyst and in the lower left-hand corner
  - Pre-Dilution (Pre-Dil)
- o One stack of 200 µL nested tips, 8 wafers per stack, no cover
- o One clean tip wafer
  - **NOTE:** The tip wafer is a clean wafer <u>without tips</u> from a 200  $\mu$ L or 50  $\mu$ L nested tip stack
- Two foil seals

**NOTE:** A full list of consumables required for this procedure can be found in **Appendix 1** 

#### 6.2. Tecan Fluent Startup and Platform Initialization

**NOTE:** Complete the following actions prior to thawing the biological samples as a conservative measure in case unforeseen technical issues arise with the

instrument. This approach will avoid compromising the biological samples. If multiple assays are performed within a day, the following steps only need to be completed before the first sample preparation of the day.

- 1. Startup of robot
  - o Turn on computer connected to the Tecan Fluent robot
    - Wait to open the Fluent software until all hardware is turned on
  - Turn on Fluent robot (Myrius box)
  - Turn on power strip connected to the Thermal Magnetic Shaker (TMS)
     control box and BioShake shakers
  - o Open the FluentControl software
    - **NOTE**: Do not interrupt the software while it is loading. The driver framework window will pop up and will disappear when the software is ready
  - o When instructed, touch anywhere on the TouchTools screen
  - Log into the FluentControl software
    - Select username
    - Enter password
- If the MCA head adapter is attached to the MCA head, proceed to Step 6.2.3
   Note: If the MCA head adapter is not attached to the MCA head, follow the steps below
  - Ensure the Deck Segment System MCA is mounted to grid position 23 on the robotic deck
    - Ensure the deck segment is securely in position with the switch on the bottom of the carrier flipped to the right, locking it in place
  - Ensure Rack Head Adapter MCA is mounted on the Deck Segment System MCA with label on rack facing analyst at position 23-2
     NOTE: If this is the first method to be run on a given day, the robot will first
    - perform an initialization step. Before starting, move both robotic arms to the center of the robot, away from the photo-cleavage light source to prevent collision errors during instrument initialization
  - o From the TouchTools home screen, select
    - System care
    - Get Head Adapter
    - Press green play button to start the "Get Head Adapter" method

- The MCA will perform the action of initializing then attaching the head adapter
- After method completion, remove deck segment with MCA head adapter cradle from Fluent deck grid position 23
  - Flip the switch on the bottom front of carrier to the left
    - > This will unlock the carrier from the robotic deck allowing for front to back movement
  - Remove carrier by moving it forward and up
     NOTE: The rear plastic pin-attachments are prone to breakage if the carrier is forcefully removed
- o Install Nest Segment, 6-Position, 7mm on grid position 23 of Fluent deck
  - Ensure the rear pins of the deck segment are securely attached to the robot deck
  - Flip the switch on the bottom front of carrier from left to right to secure the carrier in place
- 3. Shaker communication test

**NOTE:** If this is the first method to be run on a given day, the robot will first perform an initialization step. Before starting, move both robotic arms to the center of the robot away from the photo-cleavage light source to prevent collision errors during instrument initialization.

- o From TouchTools home screen, select:
  - Method Starter
  - Shaker Communication Test
  - Green Play button to initiate the Shaker Communication Test Method
- The method will execute a series of tests.
- Watch each test carefully and acknowledge the completion of each test by following the robotic prompts.



- 4. Waste collection bottle
  - Connect waste collection bottle to the waste trough
    - Screw the waste bottle cap to the bottle
    - Attach the tubing from the waste trough to the bottle cap using the quick connector

**NOTE:** To allow proper venting, make sure **BOTH** barbs are securely connected in the quick release fitting. If either barb is not securely connected, the liquid waste trough may fail to drain and can overflow from the waste trough during the assay

#### 5. Inspection of gripper fingers

- Inspect gripper fingers for deformities, damage, and debris
  - If debris is present, clean gripper fingers with an alcohol wipe
  - If there are deformities or damage, do not proceed and notify your robotics specialist or contact SomaLogic tech support
- Check leveling of the RGA fingers
  - Gently move RGA to an unobstructed (i.e., clear of labware) carrier position with a flat surface
  - Gently pull RGA head down until fingers are just above the flat surface
    - > If both fingers are parallel with surface, manually raise the RGA head
    - If fingers are not parallel with the surface, see Appendix 2 for instructions on how to level the RGA gripper fingers

#### 6.3. Reagent and Sample Preparation

- 1. Electronic Documentation
  - Obtain a copy of the SomaScan Assay 7K Kit Urine Sample Dilution Workbook (D0005014)
  - Obtain the protein quantification results from the micro BCA assay
  - Obtain a copy of the SomaScan Assay 7K Kit Workbook (D0004623)
  - o Save each workbook with the Study ID
    - The study ID should consist of a letter code for your institution followed by a digit year, a consecutive number, and set number
      - Example: SL-21-002\_Set001
- 2. Obtain the following reagents:

Reagent/Kit	Amount	Item/Part Number	Storage Location	
Assay Buffer (AB)	1 each	651-00125	4 °C storage	
Kit, SomaScan Assay 7K, ambient	1 each	899-00042	Ambient room	
components			temperature	
Kit, SomaScan Assay 7K, 4 °C	1 each	899-00043	4 °C storage	
components	reacii	099 00043	4 C storage	
Kit, Single Dilution SomaScan				
Assay 7K20 °C Single Dilution	1 each	899-00050	-20 °C storage	
components				
Kit, Single Dilution SomaScan	1 each	899-00051	-90 °C storage	
Assay 7K80 °C CSF components	reacti	099-00001	-80 °C storage	

**NOTE:** See **Appendix 3** at the end of this document for a list of subcomponents associated with the Urine Kit (900-00026).

- Record the lot numbers of all the kit components and the Assay Buffer in the Reagents tab of the assay workbook (D0004623)
- Store the Single Dilution SOMAmer-Bead Plate (part of Single Dilution SomaScan Assay 7K, -20 °C components) and all components of the SomaScan Assay 7K, 4 °C kit at room temperature until needed
   NOTE: It may take up to 30-minutes for the Single Dilution SOMAmer-Bead Plate to completely thaw.

#### 3. Prepare reagents

- o Thaw sample diluent
  - Place the Urine Diluent (**D-U**) in the 25 °C water bath
  - Set a timer and thaw for at least 20 minutes
- Thaw 10x Slide Block (3-B) Aliquot
  - Place the 10x Slide Block (3-B) Aliquot in the 25 °C water bath
  - Retrieve during Auxiliary Reagent preparation (Step 7)
- o Thaw 100x Tag Reagent (1-T) Aliquot and Tag Diluent (1-D) Aliquot
  - Place the 100x Tag Reagent (1-T) Aliquot in the 25 °C water bath
  - Place the Tag Diluent (1-D) Aliquot in the 25 °C water bath
  - Retrieve at the start of the SomaScan Assay 7K Kit Single Dilution Assay Instructions protocol (D00004923)

- 4. Retrieve and prepare the samples
  - Retrieve the Matrix sample rack containing the randomized samples (85 research samples) with associated protein concentrations
    - Add the urine controls from the -80 °C Kit (899-00052)

Calibrator Replicates	QC Replicates	Blank Replicates
(651-00102)	(651-00103)	(650-00033)
5	3	3

- The control samples should be distributed across the number of columns of samples analyzed
  - As general guidance, limit the number of controls per column and per row to one
  - > Do not place the replicate controls directly adjacent
- Update the sample plate map in the assay workbook (D0004623) to include the control samples
- o Thaw the samples
  - Remove Matrix sample rack lid
  - Place in the 37 °C incubator
  - Set a timer and thaw samples for at least 30 minutes

#### 6.4. SomaScan 7K Sample Preparation: Urine

- 1. Preparing Samples
  - After the 30-minute sample thaw, remove the sample rack from the 37 °C incubator
    - Ensure samples are completely thawed by inspecting the sample tubes
      - If not thawed, return rack to the 37 °C incubator and thaw for additional 5 minutes
  - o Check samples for dissolution of cryo-precipitates:
    - If cryo-precipitates are still present at the end of the thaw, make a sample note in the assay workbook (D0004623)
  - o Centrifuge sample rack at 1000 × g for 1 minute
  - Remove sample tube caps using a decapper
    - Confirm that no sample is present in caps
      - If sample is present in the caps

- Replace caps on tubes
- o Centrifuge sample rack at 1000 × g for 1 minute
- 2. Prepare the Pre-Dilution (Pre-Dil) Plate

NOTE: Populating the sample dilution workbook (D0005014) with the sample protein concentrations and the addition of Assay Buffer may be completed during the 30-minute sample thaw (Step 6.3.4)

- o Obtain the micro BCA protein concentration results and paste the following information into the sample dilution workbook (D0005014)
  - Column B Sample ID
  - Column C Concentration (µg/mL) **NOTE:** Populate 115 µg/mL for the concentration of the controls.
- Obtain the Pre-Dilution (Pre-Dil) Plate prepared in Step 6.1
- Obtain 86 mL of Assay Buffer
  - According to the volumes indicated in the sample dilution workbook (D0005009), add Assay Buffer (CRITICAL VOLUME) to the corresponding wells of the Pre-Dilution (Pre-Dil) Plate

NOTE: If the calculated protein concentration for a urine sample is <83.35 µg/mL, the sample will not be pre-diluted and no Assay Buffer (AB) will be added to this sample.

 According to the volumes indicated in the sample dilution workbook, add sample (CRITICAL VOLUME) to the corresponding wells of the Pre-Dilution (Pre-Dil) Plate

NOTE: If the calculated protein concentration for a urine sample is <83.35  $\mu$ g/mL, **130 \muL** of neat sample will be added.

- Rinse the tips by gently pipetting up-and-down five times
- Mix diluted samples by gently pipetting up-and-down five times with a 200µL multichannel pipette set to 100 µL
- o Centrifuge plate at 1000 × g for 1 minute
- 3. Prepare Dilution Plate Dil 1 (**D1**)
  - After thawing the Urine Diluent (D-U)
    - Remove sample diluent from the water bath
    - Ensure diluent is completely thawed
    - Invert diluent several times to thoroughly mix, avoid creating bubbles

- Obtain the Dil 1 (D1) Plate prepared in Step 6.1
- Add 108 μL of Pre-Dilution (Pre-Dil) diluted sample (CRITICAL VOLUME) to all wells of the Dil 1 (D1) Plate
- Add 12 μL of diluent (CRITICAL VOLUME) to all wells of the Dil 1 (D1) Plate
   NOTE: Final concentration will be 75 μg/mL or 90% of calculated
  - Rinse the tips by gently pipetting up-and-down five times
  - Mix diluted samples by gently pipetting up-and-down five times with a 200μL multichannel pipette set to 60 μL
- o Centrifuge plate at 1000 × g for 1 minute
- o Foil seal and set Dil 1 (D1) plate aside until further instructions

#### 4. Fluent preparation

Setup refillable reagent trough

**NOTE:** Refillable reagent trough setup can be completed during the 30-minute sample thaw (**Step 6.3.4**)

- Fill refillable trough bottle with 1 L of Assay Buffer (AB)
- Place bottle base on left rail-pin 7
- Place trough on deck position 16-4
- Route the tubing so it is positioned in front and under deck carriers 2 and 9, just above the TouchTools screen
- Insert refillable trough bottle into the base. The bottle will click into place when properly secured
- Ensure the trough starts to fill with Assay Buffer and verify there are no large bubbles in the tubing
  - If the trough is not filling on its own, it may be necessary to prime the tubing by lowering the Refillable Regent Trough to a position lower than the Refillable Regent Base to remove any large bubbles that may be present and initiate buffer flow. Return the trough back to deck position 16-4

#### 5. Method selection

- From the TouchTools home screen select the SomaScan Sample Prep
   Urine method and press the green play button
- 6. Method initiation

- When prompted on the Method title screen, acknowledge the RUO statement
- When prompted enter the Study ID
  - Example: SL-21-002\_Set001

#### 7. Fluent deck setup

- Obtain the consumables prepared in Step 6.1
  - One stack of 200 μL nested tips, 8 wafers per stack, no cover
  - One foil seal
  - One clean tip wafer
- Obtain the Single Dilution SOMAmer-Bead Plate and carefully remove the foil seal

**NOTE:** Make sure to hold the plate securely while removing the seal to avoid splashing.

NOTE: <u>DO NOT</u> place the v4.1 SOMAmer-Bead Plate into a thermal adaptor

o Obtain the Dil 1 (**D1**) Plate and carefully remove the foil seal.

**NOTE:** Make sure to hold the plate securely while removing the seal to avoid splashing

 When prompted by the robotic script, add the following items to the Fluent deck

Deck Position	Item
Left rail-pin 7	Refillable Regent Base and Bottle*
2-2	Stack 200 µL Nested Tips (8 wafers per stack, no lid)
16-4	Refillable Regent Trough filled with Assay Buffer
	( <b>AB</b> )*
30-4 (waste trough)	Clean Tip Wafer
23-5	Dil 1 ( <b>D1</b> )
23-6	Sample Rack (no lid, lock tabs facing analyst)

<sup>\*</sup>Refillable reagent base, bottle, and trough should already be on the robot deck as prepared above in **Step 6.3.4 Fluent Preparation** 

Verify deck layout

<sup>\*\*</sup>Ensure the v4.1 Single Dilution SOMAmer-Bead Plate is completely thawed before placing on the robotic deck

- Select **Continue** within the TouchTools prompt to initiate Single Dilution
   SOMAmer-Bead Plate preparation steps and sample addition
  - Carefully watch for short volume transfers, bubbles, bead aspiration or any other abnormality during robotic pipetting
  - Document any observation in the Plate Map tab of the SomaScan Assay
     7K Workbook
- When prompted carefully remove the v4.1 Single Dilution SOMAmer-Bead
   Plate from the robot deck

#### 6.5. Binding Reaction

- 1. Binding setup
  - Seal the v4.1 Single Dilution SOMAmer-Bead Plate with the foil seal, ensuring that each well is tightly sealed.
  - o Place the sealed plate in the binding shaker and confirm the settings:

Parameter:	Temperature	Time	Shake Speed
Setting:	28 °C	3 hours	850 rpm
		30 minutes	

- o Turn the shaker on and confirm the shaker reaches **850 rpm**
- Record the following in the Plate Map tab of the SomaScan Assay 7K
   Workbook
  - Binding start time
  - Calculated binding end time (start time + 3.5 hours)

#### 6.6. Robot Cleanup

- 1. Fluent cleanup
  - Remove sample rack from deck and retain to create the Sample Import sheet within the SomaScan Assay 7K Workbook.
  - Clear deck
    - Remove remaining tips
    - Remove and discard sample dilution plate and tip wafer from the waste trough into a biohazardous waste bin
    - Leave the Tecan powered on and the FluentControl running
    - Recommendation: Wipe down deck with a disinfectant cloth

#### 6.7. Sample Plate Map

- A Sample Import Sheet is required for SomaLogic to normalize and calibrate
  the data set. The Sample Import Sheet is part of the Single Dilution SomaScan
  Assay 7K Workbook and is being populated through information entered in the
  Reagents and the Plate Map tab of the workbook.
- Complete the Assayed Sample List tab in the following way (all fields are case sensitive):
  - The following information is required for SomaLogic control samples (part of the kit):
    - Sample Type
      - Calibrator
      - > QC
      - Buffer
    - Studyld
      - Control
    - Barcode
      - Lot number of the control (from label)
  - o The following column must be blank for SomaLogic control samples:
    - SampleId
  - The following information is <u>mandatory</u> for customer samples:
    - Studyld
    - SampleId
    - Optional1
      - Copy and paste the protein concentrations from column C of the Urine Sample Dilution Workbook (D0005014)
  - o Optional Information for customer samples ONLY:
    - TimePoint
    - SampleGroup
    - SampleDescription
    - SampleMatrix
    - Volume Submitted
    - Optional1
- 3. Complete the Plate Map tab of the SomaScan Assay 7K Workbook

**NOTE:** The Plate Map tab in the workbook is intended to be used for tracking information during the hybridization and readout part of the SomaScan Assay 7K Kit for Urine, as well as to convey information about the sample preparation and the assay on a per-well basis. Some information in the table is populated through the information entered in the Assayed Sample List tab.

- If samples clogged or had low volume, select the checkbox in the column "Clogged" or "Low Volume"
  - The selection will show up in the Sample Comments column of the plate map

# 7. SomaScan Assay 7K Kit - Single Dilution Assay Instructions: Auxiliary Reagent Prep (Duration: ~10 min)

#### 7.1. Auxiliary Preparation

**NOTE:** The following plates may be prepared in any order and completed prior to the start of the single plate SomaScan assay

- 1. Magnetic Bead (M-B) Plate preparation
  - Obtain an Abgene Plate and label as Magnetic Beads (M-B)
  - o Retrieve the Magnetic Beads from the 4 °C kit
  - Vortex Magnetic Beads (M-B) for at least 1 min to ensure proper bead resuspension
  - o Immediately pour vortexed beads into a reagent reservoir
  - Pipette 75 μL Magnetic Beads (M-B) (CRITICAL VOLUME) into wells of Magnetic (M-B) Bead Plate
  - Seal plate with a foil seal
  - Store plate at 4 °C
- 2. MB Prep Buffer (M-P) Plate preparation
  - Obtain a Nunc round bottom plate and label MB Prep Buffer (M-P)
  - Pour the MB Prep (M-P) Buffer into a reagent reservoir
  - o Pipette 100 μL MB Prep (M-P) Buffer into wells of MB Prep (M-P) Buffer Plate
  - Seal plate with a foil seal
  - Store plate at 4 °C
- 3. 10x Slide Block (**3-B**) Plate preparation

- Obtain a V-bottom plate and label Slide Block (3-B)
- o Retrieve 10x Slide Block (3-B) aliquot from the water bath
- o Vortex for approximately 15 sec
- o Quick spin in a microfuge
- o Pour into a reagent reservoir
- o Pipette 15 µL 10x Slide Block (3-B) into wells of Slide Block (3-B) Plate
- Seal plate with a foil seal
- Store plate at 4 °C
- 4. MB Block (2-B) Plate Preparation
  - Obtain a V-Bottom Plate and label MB Block (2-B)
  - o Retrieve the MB Block (2-B) aliquot from 4 °C kit
  - o Pour into a reagent reservoir
  - o Pipette **30 μL** MB Block (**2-B**) into wells of MB Block(**2-B**) Plate
  - o Seal plate with a foil seal
  - Store plate at 4 °C

#### 7.2. Sample Documentation

- 1. Sample preparation and well-specific notes
  - Update Assay Overview tab of the workbook
    - Transfer sample preparation notes to Section 2 of Assay Overview tab
    - o If there were no sample preparation notes, enter "None"
  - Update the electronic Plate Map of the workbook
    - Add all well-specific notes to the Plate Map tab of the workbook
    - Save the workbook file

### 8. Reference Documents

Document ID	Document Title
D0004350	The SomaScan Assay: Recommended Sample Handling and
D0004330	Processing for Core Sample Types
D0004619	SomaScan Assay 7K Kit - Equipment List
D0004621	Fluent Operation and Maintenance Overview
D0004623	SomaScan Assay 7K Kit - Workbook
D0004919	SomaScan Assay 7K Kit - Overview and Introduction
D0004921	SomaScan Assay 7K Sample Preparation Urine Kit- User Manual
D0004923	SomaScan Assay 7K Kit - Single Dilution Assay Instructions
D0004924	SomaScan Assay 7K Kit - Single Dilution Consumable List
D0005009	Urine Pre-processing – User Manual
D0005011	SomaScan Assay 7K Urine Kit - Experienced User Checklist
D0005014	SomaScan Assay 7K Kit - Urine Sample Dilution Workbook

### **Appendix 1: Consumable List**

Single Dilution SomaScan 7K Sample Preparation, Consumables List				
Common Name	<b>Amount Consumed</b>	Amount Needed		
Matrix Tubes with Rack	85 each	85 each		
200 µL Nested Tips	2 wafers	8 wafers		
Abgene Plate	1 each	1 each		
2 mL Deep-Well Plate	1 each	1 each		
Nunc Round-Bottom Plate	2 each	2 each		
Nunc V-Bottom Plate	2 each	2 each		
Foil Seals	6 each	6 each		
20 μL Tips for multi-channel pipette	1 box	1 box		
200 µL Tips for multi-channel pipette	3 boxes	3 boxes		
Tip Wafer	1 each	1 each		

# **Appendix 2: Instructions for Adjustment of RGA Gripper Fingers**

**NOTE:** Additional details can be found in the Fluent Operation and Maintenance Overview document (D0004621)

NOTE: Ensure the robot has been initialized before attempting this procedure

- 1. Remove one of the nest carrier deck segments and replace it with the flat deck segment
- 2. Manually move RGA head over flat deck segment and lower until fingers are just above surface
- 3. Take instrument out of Zero-Gravity mode
- 4. Loosen screws on gripper fingers
- 5. Using the Move tool in FluentControl, step RGA down until Z = 0.0
- 6. Adjust the gripper fingers so they align flush with the carrier
- 7. Tighten the screws on both gripper fingers (do not overtighten)
- 8. Using the Move tool, put RGA back in to Zero-Gravity mode
- 9. Manually raise RGA head

## **Appendix 3: Components Associated with the Urine Kit**

Kit Part Number	Name	Subcomponent Part Number	Subcomponent Name	Storage Temperature
900-	SomaScan	899-00042	SomaScan Assay 7K,	Ambient
00026	Assay 7K -		ambient components	(+10 to +30 °C)
	Urine Kit	520-00010	Abgene Storage plate	Ambient
			(3 each)	(+10 to +30 °C)
		899-00043	SomaScan Assay 7K,	+4 °C
			4C components	(+2 to +8 °C)
		899-00050	SomaScan Assay 7K, -	-20 °C
			20C Single Dilution	(-10 to -30 °C)
			components	
		899-00052	SomaScan Assay 7K, -	-80 °C
			80C Urine components	(-70 to -90 °C)
		651-00125	Assay Buffer, 1000 mL	+4 °C
				(+2 to +8 °C)
900-	Single	899-00047	SomaScan Assay 7K,	Ambient
00029	Dilution		ambient components,	(+10 to +30 °C)
	SomaScan		no slides	
	Assay 7K -	520-00010	Abgene Storage plate	Ambient
	Urine Kit,		(3 each)	(+10 to +30 °C)
	no slides	899-00043	SomaScan Assay 7K,	+4 °C
			4C components	(+2 to +8 °C)
		899-00050	Single Dilution	-20 °C
			SomaScan Assay 7K, -	(-10 to -30 °C)
			20C components	
		899-00052	SomaScan Assay 7K, -	-80 °C
			80C Urine components	(-70 to -90 °C)
		651-00125	Assay Buffer, 1000 mL	+4 °C
				(+2 to +8 °C)

Component Part Number	Component Name	Subcomponent Part Number	Subcomponent Name	Code
899-00042	SomaScan	651-00126	MB Prep Buffer, 11 mL	M-P
	Assay 7K,	651-00033	2x Hybridization Buffer, 3.5	3-H
	ambient		mL	
	components	300-00008-012	Microarray, 8x60k, 8 per,	
			AMADID#086332, 12 pk	
899-00043	SomaScan	651-00003	Quench Buffer, 85 mL	1-Q
	Assay 7K, 4C	651-00005	Elution Buffer, 20 mL	2-E
	components	651-00007	Photo-Cleavage Buffer, 40	1-PC
			mL	
		651-00030	MB Block, 3.5 mL	2-B
		651-00031	Magnetic Beads, 8.5 mL	M-B
		651-00035	Tag Diluent, 40 mL	1-D
		651-00074	MB Wash Buffer 20%, 85 mL	2-W
899-00050	SomaScan	651-00123	SOMAmer-Bead Plate Single	
	Assay 7K, -		Dilution v4.1	
	20C single	651-00008	100x Tag Reagent, 460 uL	1-T
	dilution	651-00009	10x Slide Block, 1.8 mL	3-B
	components			
899-00052	SomaScan	651-00102	v4.1 Urine Calibrator, 120 uL	
	Assay 7K, -	651-00103	v4.1 Urine Calibrator, 120 uL	
	80C urine	650-00033	Blank, 140 uL	
	components	651-00073	Urine Diluent, 5 mL	D-U
899-00047	SomaScan	651-00126	MB Prep Buffer, 11mL	M-P
	Assay 7K,			
	ambient			
	components,			
	no slides			