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SomaScan® 7K Assay v4.1: Urine Pre-Processing User Manual

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This user manual describes the steps for the pH adjustment, buffer exchange and protein quantification of human biobank urine samples prior to use in the Urine Sample Preparation protocol and Single Dilution SomaScan Assay 7K and is applicable to kit part number: 900-00026

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1. Introduction

Urine samples require pH normalization, buffer exchange, and total protein concentration measurements before analysis in the SomaScan Assay 7K Single Dilution Kit.

This procedure describes the pre-processing steps required to pH normalize and buffer exchange up to 96 human urine samples, as well as considerations and recommendations for protein quantification. Of the 96 pre-processed urine samples, up to 85 can be tested in a single dilution SomaScan Assay 7K kit (part number 900-00026); therefore, an additional 11 urine samples can be processed using this protocol. This procedure yields the pH adjusted (Tris-treated) and buffer exchanged urine samples in matrix tubes that will be used during the sample preparation procedure (D0004921). Urine samples may be pre-processed at any time prior to the start of the SomaScan Assay sample preparation.

The measurement of the total protein concentration in the urine samples must be done using the pH-adjusted and buffer exchanged urine as an input to the micro BCA assay. Due to the large total protein concentration differences observed for urine samples, SomaLogic recommends a 20-fold dilution of the pH-adjusted and buffer exchanged urine as the micro BCA input sample. The preparation of the 20-fold diluted samples is described as an optional step in this procedure.

2. Initial Setup Requirements

2.1. Required Equipment*

Equipment	Suggested Part Number	Setting
Digital Incubator	VWR, 414005-130 (or equivalent)	37 °C
Centrifuge	Eppendorf, Centrifuge 5804, keypad, non-refrigerated, with rotor A-2-DWP, US 3 (or equivalent)	1000 x g, 2-min

* The listed vendors for these items are suggestions only. Other brands/models with equivalent specifications are acceptable.

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2.2. Required Reagents and Storage Conditions

Reagent	Part Number	Storage Temperature
Assay Buffer (**AB**)	651-00125	+4 °C (+2 to +8 °C)
1M Tris, pH 8.0 (Tris [hydroxymethyl] aminomethane)	Sigma Aldrich, 648314-100mL	Ambient (+10 to +30 °C)
diH_20	N/A	Ambient (+10 to +30 °C)
Zeba™ Spin Desalting Plates - 2	ThermoScientific #89807	+4 °C (+2 to +8 °C)

2.3. Consumables Required

Consumable	Part number	Quantity
Nunc Round Bottom Plate	ThermoFisher Scientific #267334	1
Blue Collection Plates (Supplied in the Zeba Kit)	ThermoFisher Scientific #89808	2
Opaque Collection Plates (Supplied in the Zeba Kit)	ThermoFisher Scientific #89808	2
Matrix Tubes	ThermoFisher Scientific #3744	96
Foil Seal	Bio Rad #MSF-1001	1

3. Procedure for pH Adjustment and Buffer Exchange of Urine Samples (Duration: ~1.5 h)

3.1. Consumable Preparation

1. Obtain one foil seal
2. Obtain one Matrix rack (96 tubes)
3. Obtain two opaque collection plates and label:
    - Wash
4. Obtain two blue collection plates and label as follows on the front and left-hand side (ensure to maintain proper alphanumeric orientation):
    - Sample collection

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5. Obtain one Nunc round bottom plate and label as follows on the front and left-hand side with the notch facing towards the analyst and in the lower left-hand corner:
    - pH Adjustment (**pH Adj**)

3.2. Reagent and Sample Preparation

1. Obtain the following reagents:

Reagent	Amount	Storage Location
Assay Buffer (**AB**)	73 mL	4 °C storage
1M Tris, pH 8.0 (Tris [hydroxymethyl] aminomethane)	2 mL	Ambient room temperature
diH_20	25 mL	Ambient room temperature
Zeba™ Spin Desalting Plates	2 each	4 °C storage

    - Allow the Zeba plates and Assay Buffer (**AB**) to equilibrate to room temperature prior to use in this protocol
2. Retrieve and thaw urine samples
    - Retrieve up to 96 urine samples from -80 °C storage
    - Place samples in the 37 °C incubator for at least 30 minutes
    - While samples are thawing prepare the Zeba plates
3. Prepare the Zeba plates
    - Obtain the previously labeled wash plates
    - Wash the Zeba plates
        - Remove the Zeba plate seals
            - ➢ Use the tab to remove the sealing material from the bottom of the plates
            - ➢ Place each Zeba plate on top of a wash plate
            - ➢ Remove the sealing material from the top of the plates
        - Place the stacked Zeba/wash plates into a centrifuge
            - ➢ Centrifuge at 1000 × g for 2 minutes to remove the storage buffer
            - ➢ Discard the flow-through and retain the wash plates for the remaining Assay Buffer (**AB**) washes
        - Perform four Assay Buffer (**AB**) washes
            - ➢ Add **250µL** of Assay Buffer on top of the resin bed

- Centrifuge at 1000 × g for 2 minutes
- Discard the flow-through
- Repeat for a total of 3 washes
- On the fourth wash, add **250µL** of Assay Buffer (**AB**) on top of the resin bed, but wait to spin through until the completion of pH adjustment **Step 3.2.4**

4. pH adjustment
   - Retrieve the following reagents, consumables, and samples
     - 1 M Tris pH 8.0 – 2 mL
     - pH Adjustment Plate (**pH Adj**)
     - Thawed urine samples
   - Make the pH Adjustment (**pH Adj**) Plate
     - Open the sample tubes
     - Carefully mix samples by pipetting slowly up-and-down 5 times
     - Adjust pH of the urine samples
       - Add **216 µL** (**CRITICAL VOLUME**) of sample into the pH Adjustment (**pH Adj**) Plate
       - Add **9 µL** (**CRITICAL VOLUME**) of 1 M Tris pH 8.0
         NOTE: Final Tris concentration is 40 mM
       - Rinse the tips by gently pipetting up-and-down 5 times
       - Mix Tris-treated samples by gently pipetting up-and-down 3 times with a 200 µL multichannel pipette set to 112 µL
5. Buffer exchange
   - Obtain the Zeba plates
   - Place each Zeba plate on top of a wash plate to complete the 4th Assay Buffer (**AB**) wash
     - Centrifuge at 1000 × g for 2 minutes
     - Discard the wash plates with the flow-through
   - Obtain the previously labeled sample collection plates
   - Place each Zeba plate on top of a sample collection plate
     - Ensure proper alignment of the alphanumeric indices on the plates
   - Add **100 µL** of the Tris-treated sample to the center of the resin bed of the first Zeba plate

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- To expel the entire sample, carefully touch pipette tip to the side of the well avoiding touching the resin

- Add **100 µL** sample to the center of the resin bed of the second Zeba plate
- Centrifuge the plates
  - 1000 × g for 2 minutes
- After centrifugation, ensure all sample has been spun through the Zeba plates
  - Carefully retrieve the sample collection plates and save the processed urine samples
  - Discard the Zeba plates into the appropriate biohazard waste stream

6. Sample Matrix rack
   - Obtain the two sample collection plates containing processed urine
   - Obtain the Matrix rack with tubes

     NOTE: Matrix tubes must be associated with the proper sample information
   - Combine like samples
     - Ensuring proper alphanumeric plate orientation
       - Transfer all the volume (~**100 µL**) of sample from the first collection plate into the tubes of the Matrix rack
       - Transfer all the volume (~**100 µL**) of sample from the second collection plate into the tubes of the Matrix rack

         NOTE: Final processed urine sample volume should be ~200 µL.
     - Discard the empty sample collection plates into the appropriate biohazard waste stream
     - It is recommended to make the 20-fold Dilution Micro BCA Plate **Step 3.3**

       NOTE: Complete this step before storing the samples at -80 °C.
     - Cap the tubes in the Matrix rack and store at -80 °C until urine sample preparation procedure D0004921

3.3. Considerations/Recommendations for the Micro BCA

1. The 20-fold Dilution Micro BCA Plate:
   - pH adjusted and buffer exchanged urine samples should be diluted 1 to 20 in water as the initial input for a micro BCA assay
     - Recommended storage: 4 °C for up to 1 week
     - The volume of 1 to 20 diluted urine is enough for two micro BCA assays or subsequent dilutions, if needed

- Directions on how to make a 20-fold Dilution Micro BCA Plate can be found in **Appendix 1**

2. Highly concentrated urine samples
   - It might be necessary to dilute the samples more than 20-fold to assure that determined protein concentrations fall within the working range of the micro BCA assay
     - If additional dilutions are required, dilute the urine sample in 0.05% assay buffer (i.e., Assay Buffer [**AB**] diluted 1 to 20 in di-water)
     - 0.05% assay buffer is not supplied in the kit; however, directions on how to make 0.05% assay buffer can be found in **Appendix 2**
3. The micro BCA assay requires the preparation of a protein standard curve
   - Prepare the standard curve with the BSA provided in the micro BCA kit using 0.05% assay buffer

4. Reference Documents

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

Appendix 1: 20-fold Dilution Micro BCA Plate

NOTE: Performing protein quantification using a micro BCA assay on the pH adjusted and buffer exchanged urine samples is required prior to performing the urine sample preparation procedure (SomaScan Assay 7K Sample Preparation Urine Kit- User Manual D0004921).

NOTE: Diluting the urine samples 20-fold for micro BCA testing is recommended, due to possible interfering substances that may be present in the samples.

NOTE: Creating the 20-fold dilution micro BCA plate at the end of the pH adjusted and buffer exchange procedure will prevent an additional freeze-thaw cycle of the urine samples.

1. Prepare the 20-fold dilution micro BCA plate
2. Obtain the following regents and consumables:
   - diH20 - 25 mL
   - Nunc round bottom plate - 1 each
3. Label the front and left-hand side with the notch facing towards the analyst and in the lower left-hand corner: 20-fold Micro BCA
4. Matrix rack with prepared urine samples from Step 3.2.6
5. Make the 20-fold Micro BCA Plate
   - Add **247 µL** (**CRITICAL VOLUME**) of diH_20 into the 20-fold Micro BCA plate
   - Add **13 µL** (**CRITICAL VOLUME**) of sample from the Matrix Rack
     - Rinse the tips by gently pipetting up-and-down five times
     - Mix 20-fold diluted samples by gently pipetting up-and-down three times with a 200 µL multichannel pipette set to 130 µL
   - Foil seal 20-fold micro BCA plate and store at 4 °C until micro BCA processing

     NOTE: 20-fold micro BCA plate can be stored up to 1 week at 4 °C
   - Cap the tubes in the Matrix rack and store at -80 °C until Urine sample preparation procedure D0004921
   - Perform the micro BCA assay per manufacturer instructions and by adhering to the considerations and recommendations in **Section 3.3**
     - Use Micro BCA Protein Assay Kit (ThermoFisher Scientific, P/N: 23235)
   - Retain and associate the protein concentration results for the urine samples

Appendix 2: Making 0.05 % Assay Buffer

- Obtain the following regents:
  - diH_20
  - Assay buffer
- To make 50 mL of 0.05% assay buffer, for example:
  - Add **2.5 mL** Assay Buffer (**AB**) to **47.5 mL** di-water

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