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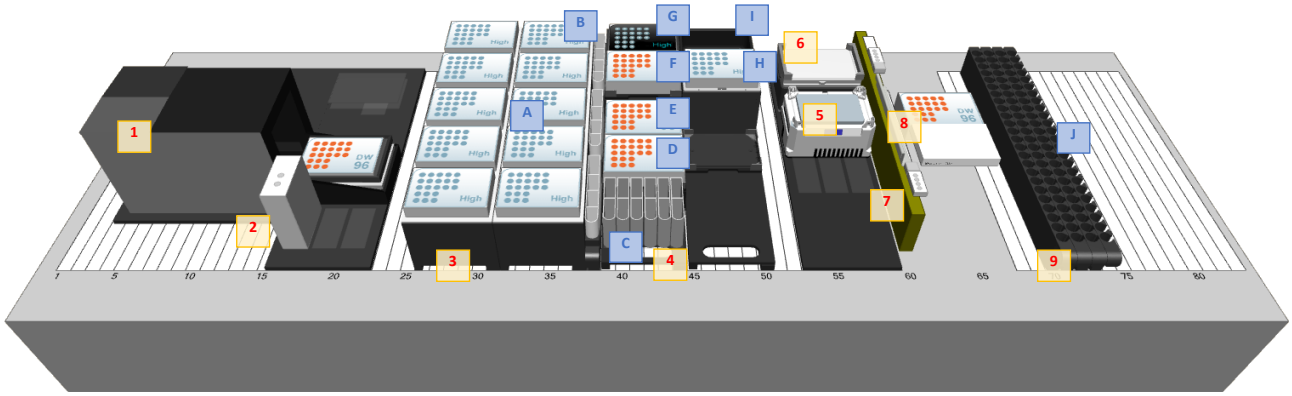
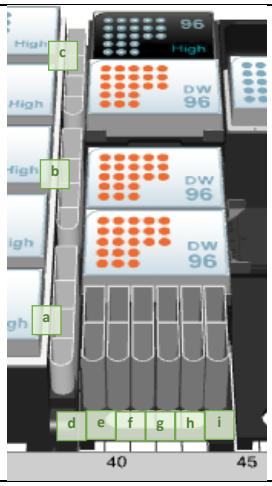
Supplementary Protocol - Automated Procedure for Saliva/Swab RNA Purification and Concentration

Product # 69300 & # 72000 (Automation Accessories Kit)

Materials and Deck Setup



Hamilton STAR Vantage		Consumables		Quantity
1	[MPE]2 positive pressure system	A	1000 µL CO-RE Disposable Tips	≥ 4 × 96
2	CO-RE Plate Gripper	B	Reagent Troughs (120 mL)	3
3	Tip Carriers and Racks (x2)	C	Reagent Troughs (60 mL)	6
4	Reagent carriers and racks (x5)	D	2.0 mL Deep Well Lysate Plate (from Norgen Automation accessory kit # 72000)	1
5	Heater/cooler	E	96-Well Isolation Plate (from Norgen # 69300) on top of the Adaptor Plate (from Norgen Automation accessory kit # 72000)	1
6	Heater/shaker	F	1.1 mL Elution plate (from Norgen kit # 69300)	1
7	Calibrator tips	G	Tip Adaptor	N/A
8	Tip waste	H	Tip Isolator	N/A
9	Sample carriers	I	Gravitational Waste	N/A
		J	Saliva/Swab collection tubes	≤ 96



	Reagents	Quantity*
a	-	
b	-	
c	Wash Solution A	90 mL
d	PBS Buffer (1X)	18 mL
e	Lysis Buffer A	50 mL
f	Ethanol (96-100%)	50 mL
g	WN Solution	50 mL
h	Elution Solution A	10 mL
i	-	

**Quantity needed for 96 isolations*

Customer Supplied Equipment and Reagents

- Hamilton Automation System and consumables
- 1X PBS pH 7.4
- Ethanol (96-100%)
- **Norgen Biotek's Saliva/Swab RNA Purification 96-Well Kit Automation Accessories (Cat. No. 72000)**
    - **2.0 mL Deep Well Lysate Plate x2**
    - **Adaptor Plate x2**
    - **Norgen Biotek's Sputum Liquification Buffer (Cat. No. 28289)**

Procedure

Sample Preparation on the Lab Bench

A. Saliva Samples

1. Add 400 µL of Sputum Liquification Buffer to the preserved saliva samples, then incubate at 95°C for 60 minutes in a water bath.
2. Invert to mix after incubation, remove caps and load the tubes onto the sample carrier.

B. Swab Samples

1. Remove caps and load tubes onto the sample carrier.

Sample Preparation on the Hamilton STAR Vantage

Instrument Set-Up & Initialization

1. Turn on the instrument and [MPE]2 system by pressing the power button on both units. Open the current method using the "Method Editor" program on the computer.
2. Load the pipette tips and plates according to the diagram in the above section Materials and Deck Setup.
   Optional: You may view the Deck Layout by selecting "view > System Deck" or pressing Ctrl+Shift+Y. Ensure the layout on the system deck matches the actual deck setup on the instrument.
3. On the Method Editor program, press F5 to open the "Run Control" program. You will be notified when the program connects to the system. Press the green play button to start the running the method (Figure 1).
4. The Vantage will perform an initialization procedure to ensure that the instrument is functioning properly.
5. Once finished initializing, the program will prompt the user to enter the number of samples to run. (Figure 2).
6. The user will have to select the location of the pipette tips that are currently on the instrument.
7. The extraction method will proceed uninterrupted.

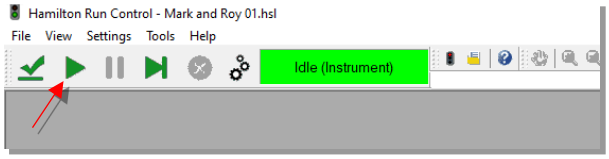


Figure 1. Run Control menu after connecting to the instrument.

Figure 2. Sample Entry and Selection.

Running the RNA Extraction Method

1. 150µL of 1X PBS is added to a 96-Well Lysate Plate. The number of wells with PBS corresponds to the number of samples input by the user.
2. To each well being used in the 96-Well Lysate Plate, 400uL of Lysis Buffer A is added.
3. 250 µL of each sample is added into the 96-Well Lysate Plate.
4. The 96-Well Lysate Plate is transferred to the heater/shaker and mixed at 1,500 rpm for 1 minute.
5. The 96-Well Lysate Plate is transferred back to its original position and 400 µL of 96-100% ethanol is added to each lysate in the plate.
6. The Isolation Plate is transferred to the [MPE]^2.

Filtration and Wash Steps

1. Each lysate is pipette mixed and then 600 µL is transferred into wells on the Isolation Plate and the [MPE]^2 is run for 3 minutes at 100 psi.
2. The remaining 600 µL of lysate is transferred into wells on the Isolation Plate and the [MPE]^2 is run for 3 minutes at 100 psi.
3. A prompt appears on the screen to ask the user to check the wells for any clogged lysate. If there is any clogging the user should select the appropriate box and the [MPE]^2 is run a second time. If there is no clogging the user selects the appropriate box, and the procedure continues.
4. 400 µL of Wash Solution WN is added to the Isolation Plate and the [MPE]^2 is run for 3 minutes at 100 psi.
5. A prompt appears on the screen to ask the user to check the wells for any clogged lysate. If there is any clogging the user should select the appropriate box and the [MPE]^2 is run a second time. If there is no clogging the user selects the appropriate box and the procedure continues.
6. 400 µL of Wash Solution A is added to the Isolation Plate and the [MPE]^2 is run for 1 minute at 100 psi. A dialogue box will appear, if there is any clogging the user should select the appropriate box and the [MPE]^2 is run a second time. If there is no clogging the user selects the appropriate box and the procedure continues.
7. Step 6 is repeated one more time.
8. The Isolation Plate remains on the [MPE]^2 for 15 minutes at 100 psi to allow the wells to dry.
9. 75 µL of Elution Solution A is added into each well and the Isolation Plate is clamped into the [MPE]^2.
10. The Elution Plate is placed onto the [MPE]^2.
11. The [MPE]^2 runs for 3 minutes at 100 psi.
12. The Isolation and Elution plates are unclamped from the [MPE]^2. The Elution Plate is ready for the user to remove for downstream analysis.

Storage of RNA
Use the provided adhesive tape to seal the 96-Well Elution Plate (Deep Well). The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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