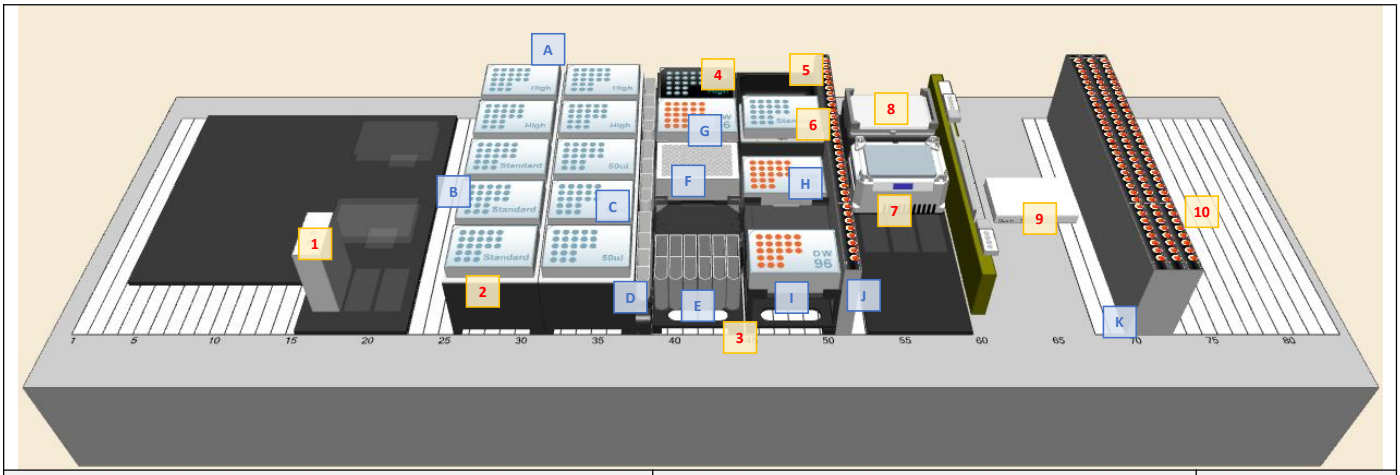
NORGEN BIOTEK CORP.

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

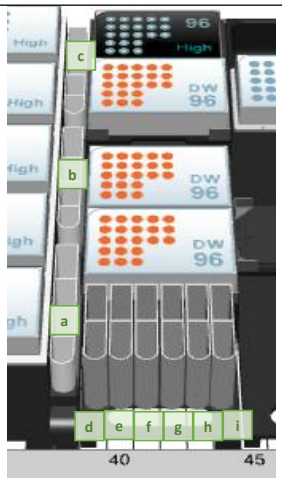
Supplementary Protocol - Automated Procedure for Stool DNA Isolation Kit (Magnetic Bead System)

Product # 63100 & # 72000 (Automation Accessories Kit)

1 Materials & Deck Setup



Hamilton STAR Vantage		Consumables		Quantity
1	CORE Plate gripper	A	1 mL tips with filter	≥ 4 × 96
2	Tip carriers and racks (x2)	B	300 µL tips with filter	≥ 1 x 96
3	Reagent carriers and racks (x2)	C	50 µL tips with filter	≥ 1 x 96
4	Tip Adapter	D	Reagent troughs (120 mL)	1
5	Gravity waste	E	Reagent troughs (60 mL)	1
6	Tip Isolator	F	Water trough	1
7	Heater / cooler	G	Final Elution Plate	1
8	Heater / shaker	H	96-well magnetic plate	1
9	Tip waste	I	96-well deep well processing plate	1
10	Sample carriers	J	Magnetic Bead Suspension tubes	4 x 800 µL
		K	1.5 mL Stool sample tubes	≤ 96



Reagents		Quantity
a	70% Ethanol	100 mL
b	100% Ethanol	40mL
c	-	-
d	Wash Solution WN	50 mL
e	Elution Buffer B	10 mL
f	-	-
g	-	-
h	-	-
i	-	-

Additional materials	Quantity

NORGEN BIOTEK CORP.

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

2 Procedure

3 Sample preparation on the lab bench

1. Add 200 µL of preserved sample to a provided Bead Tube and add 800 µL of Lysis Buffer L. Vortex briefly to mix stool and Lysis Solution.
2. Add 100 µL of Lysis Additive A and vortex briefly.
3. Secure tubes horizontally on a flat-bed vortex pad with tape, or secure the tubes in any commercially available bead beater equipment (e.g. Scientific Industries' Disruptor Genie TM).
4. Vortex for 3 minutes at maximum speed.
5. Centrifuge the tubes for 2 minutes at 20,000 × g (~14,000 RPM).
6. Transfer up to 600 µL of supernatant to a DNAase-free microcentrifuge tube (not provided).
7. Add 100 µL of Binding Buffer I, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
8. Spin the lysate for 2 minutes at 20,000 x g (~14,000 RPM) to pellet any cell debris.
9. Load the samples onto the Hamilton Vantage in the location indicated on the Deck setup for stool samples.

4 Sample preparation on Hamilton STAR Vantage

Instrument set-up & initialization

1. Turn on the instrument by pressing the power button. Open the current method using the "Method Manager" program on the computer.
2. Press the green play button to start the running the method (Figure 1).
3. The Vantage will perform an initialization procedure to ensure that the instrument is functioning properly.
4. Once finished initializing, the program will prompt the user to enter the number of samples to run (Figure 2) and the location of pipette tips in the deck (Figure 3).

Steps performed by the liquid handling machine

Sample Transfer and Lysis

5. 600 µL of each sample is aliquoted into the Sample Plate.
6. 300 µL of 96-100% ethanol is added to each sample in the Sample Plate.
7. The magnetic bead suspension is pre-mixed to ensure homogeneity, and then 20 µL is aliquoted into each sample and pipette mixed.
8. The Sample Plate is transferred to the Heater/Shaker and vortexed for 5 minutes at room temperature.
9. The Sample Plate is transferred to the magnet and waits for 1 minute for the magnetic beads to form a pellet.

Bind and Wash Steps

10. The supernatant is removed from the Sample Plate without disturbing the magnetic bead pellet and deposited in the gravity waste.
11. The Sample Plate is transferred back to the original starting position and 500 µL of Wash WN is added to each sample.
12. The Sample Plate is transferred to the Heater/Shaker and vortexed to mix for 1 minute.
13. The Sample Plate is transferred to the magnet and waits for 1 minute to pellet the magnetic beads.
14. Wash WN is removed from the samples and deposited in the gravity waste.

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

15. The Sample Plate is transferred to the starting position and 500 µL of 70% ethanol is aliquoted into each of the sample wells.
16. The Sample Plate is transferred to the Heater/Shaker and vortexed for 1 minute before being transferred to the magnet.
17. After 1 minute the ethanol is removed and dispensed into the gravity waste.
18. Steps 15-17 are repeated 2 more times for a total of 3 washes with 70% ethanol
19. The Sample Plate is transferred to the Heater/Shaker and incubated at 65°C for 5 minutes to evaporate excess ethanol.

Elution

20. The Sample Plate is returned to the original position and 75 µL of Elution Buffer B is aliquoted into each sample well.
21. The Sample Plate is transferred to the Heater/Shaker and vortexed to mix at 65°C for 10 minutes.
22. The Sample Plate is placed onto the magnet for 2 minutes.
23. The supernatant is aspirated from the Sample Plate and dispensed into the Final Elution Plate.
24. The purified DNA in the elution plate can now be removed from the Hamilton Vantage and be used for downstream analysis.

5 Notes

- DNA should be stored at -20°C after isolation is complete

Technical Support
Contact our Technical Support Team between the hours of 9:00 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

