

Product Application

Norovirus RNA Isolation from Human Feces

Isolate Norovirus RNA from human feces using Maxwell 16 Instrument and Maxwell 16 LEV simplyRNA Tissue Kit.

Kit:	Maxwell 16 LEV simplyRNA Tissue Kit (Cat. AS1280)	This protocol was developed by a customer who works for one of CRO
Analyses:	Conventional PCR and Gel electrophoresis / TaqMan Real-Time PCR	Japan and is intended for research u only.
Sample Type(s):	10% emulsion of human feces prepared by PBS	Users are responsible for determining suitability of the protocol for their application.
Input:	200μL	Further information can be found in Technical Manual #TM351, available
Materials Required:		at: <u>www.promega.com/protocols</u>
	 Maxwell 16 Instrument (Cat. #AS2000) Maxwell 16 LEV simplyRNA Tissue Kit (Cat. #AS1280) High-speed centrifuge 	

Vortex

Protocol:

Purification Step:

- 1. Prepare 10% emulsion of human feces by PBS.
- 2. Centrifuge at 14,000 xg for 3 minutes.
- 3. Transfer 200µL of supernatant to a new tube.
- 4. Add 200µL of Lysis Buffer and mix thoroughly by vortex mixer.
- 5. Transfer the entire volume of lysate to well #1 of the cartridge.
- 6. Add 50µL Nuclease-Free Water into Elution Tube, 10µL reconstituted DNase I into well #4 and put LEV Plungers onto well #8.
- 7. Load the Cartridge Rack onto the Maxwell 16 Instrument.
- 8. Begin the automated purification run.

Reverse Transcription Step:

- 1. Add 10µL of RNA sample into new tube.
- 2. Prepare 20µL ul RT reaction using SuperScript III (1µL, 200units/µL).
- 3. Incubate at 42C for 1 hour.
- 4. Heat at 99C for 5 minutes to inactivate RT and keep at 4c or on ice.

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Results:

A. Conventional PCR:

Enzyme: GoTaq DNA polymerase

Primer: COG1F, G1SKR, COG2F,G2SKR

PCR Codition: 94C, 3min. > 40 cycles (94C, 30sec. > 50C, 30sec. > 72C, 1min.) > 72C, 15min. > 10C, HOLD

Sample #	Call	G1	G2
1	G1 Positive	+	-
2	G2 Positive	-	+
3	G2 Positive	-	+
4	G2 Positive	-	+
5	G2 Positive	-	+
6	G2 Positive	-	+
7	Negative	-	-
8	G1 Positive	+	-



The result of "G1"

B. TaqMan Real-Time PCR:

Enzyme: TaqMan Universal PCR Master Mix (Applied Biosystems by Thermo Fisher) Primer: COG1F, COG1R, COG2F,COG2R

Probe: G1 - RING1-TP(a) and RING1-TP(b), G2 - RING2AL-TP

PCR Condition: 50C, 2min. > 95C, 10min. > 45 cycles (95C, 15sec. > 56C, 1min.) > 4C, HOLD

Sample #	Call	G1	G2
1	G1 Positive	<mark>33.09</mark>	No Ct
2	G2 Positive	No Ct	<mark>19.75</mark>
3	G2 Negative	No Ct	37.79
4	G2 Positive	No Ct	<mark>20.02</mark>
5	G2 Positive	No Ct	<mark>28.84</mark>
6	G2 Positive	44.68	<mark>35.47</mark>
7	Negative	No Ct	No Ct
8	G1 Positive	<mark>26.78</mark>	No Ct
C	Control	No Ct	No Ct
	Std 1	No Ct	40.97



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Std 2	40.02	37.24
Std 3	36.45	33.37
Std 5	25.41	26.55