

Read the entire Instructions for Use (IFU) and follow them carefully before performing the test.

Deviations from the given test protocol can lead to incorrect results. Good laboratory practice should be followed during the test.

Intended Use

The Norovirus *direct* RT-PCR test is an *in vitro* real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection and differentiation of norovirus (genogroup I and II) in human stool samples. The results support the diagnosis of gastroenteritis. The test is intended for use in qualified laboratories by personnel trained in molecular diagnostic techniques.

Package Content

Vials with solution A (blue lid; 1.5 mL) and solution B (yellow lid; 100 µL), each for one microtiter plate (96 well; not included), Positive Control PC (green lid; 13 µL; optional), Negative Control NC (colourless lid; 100 µL; optional), Instructions for Use.

Notes before starting

The starting material for the Norovirus *direct* RT-PCR test is **10 µL/reaction eluate from RNA extraction** isolated from human stool. This assay must not be used on a specimen directly. Appropriate nucleic acid extraction methods must be conducted prior to using this assay. RNA extraction reagents are not part of the Norovirus RT-PCR test. One Positive and one Negative Control should be included in each PCR run.

Material provided by user

- qPCR microtiter plate or reaction tubes plus lids/adhesive optical film
- qPCR instrument (e.g. LightCycler 480; Biorad CFX)
- RNA isolation kit; table centrifuge
- Adequate pipettes and sterile filter-tips for PCR testing (DNase/RNase-free)
- Disposable protective gloves, powder-free

Test procedure with RNA extraction

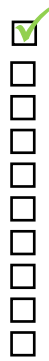
1. Thaw all reagents completely and keep them cool (+2 °C to +8 °C) directly before starting the test, use within 4 hours.
2. Add 100 µl of Solution B to the lysis buffer per extraction run in a 96-well plate format.
3. Perform RNA extraction according to your laboratory's standard procedure.
4. Pipette 15 µL of the solution A into each PCR reaction tube/well of the microtiter plate.
5. Add 10 µL of Positive Control and 10 µL of Negative Control per run in respective wells.
6. Add 10 µL of eluate from RNA extraction per remaining reaction tube/well of the microtiter plate.
7. Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided .
8. Briefly centrifuge the microtiter plates or reaction tubes.
9. Place the filled plate/reaction tubes in the qPCR cycler. Start program.

Instrument settings

Steps	Temperature [°C]	Time	Number of cycles
Reverse Transkription	55	10 min	1x
Initial denaturation	95	5 min	1x
Denaturation	95	10 sec	45x
Amplification/Elongation	60	30 sec	

Channel settings for FBC113

	Norovirus GI	IC	Norovirus GII
Reporter dye	FAM	HEX	Red 610
Colour	green	yellow-green	orange
Emission [nm]	520	560	610
Quencher	Black Hole Quencher		

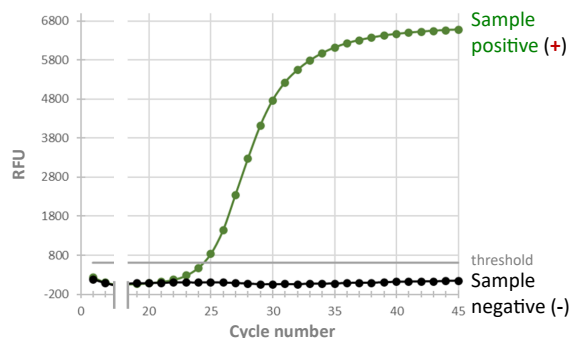


Interpretation of test results

Positive samples (+) show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value.

The results are used to identify norovirus RNA. Positive results are an indication of the presence of the pathogen. A negative result does not rule out the presence of the virus, as the results depend on correct sampling and a sufficient amount of RNA to be detected.

Norovirus GI	Norovirus GII	IC	Result	Interpretation
+	-	+/-	Valid	Norovirus GI detected.
-	+	+/-	Valid	Norovirus GII detected.
+	+	+/-	Valid	Norovirus GI and GII detected.
-	-	+	Valid	Target genes not detected.
-	-	-	Invalid	The test result can not be evaluated.



Important notes:

All samples of biological origin and used plates/swabs are to be treated as potential carriers of infectious diseases.

When working with chemicals or when handling samples of biological origin, the safety precautions of the laboratory must be observed.

Storage: -25°C -18°C Usage: +2°C +8°C

Before performing this test, read the instructions for use to familiarise yourself with the testing procedure.

You can find them on <https://frizbiochem.de/downloads/>

If you have any questions or problems, please contact service at FRIZ Biochem GmbH (<https://frizbiochem.de/get-in-touch/>).

REF FBC113

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