Application Note

Purification of microbial DNA by combining Stool Collection Tubes with DNA Stabilizer (Invitek) and the QIAamp Power Fecal Pro DNA Kit (Qiagen)

The QIAamp Power Fecal Pro DNA Kit is intended for the isolation of fresh or frozen stool samples. This application note describes how to modify the extraction kit for the isolation of samples collected and stabilized using the Invitek Stool Collection Tubes with DNA Stabilizer.

REQUIRED MATERIALS

Sample type: Human feces collected with the Stool Collection Tubes with DNA Stabilizer (Cat. no. 1038111200, 1038111300, Invitek, Fig.1), 1 g of sample needs to be collected, as described in the corresponding instructions for use.

Sample volume: 800 µl stabilized sample

Extraction Kit: QIAamp Power Fecal Pro DNA Kit (Cat. no. 51804, Qiagen)

Reagent: 2-Propanol

Equipment: Bullet Blender, or comparable bead mill homogenizer
Vortex mixer
Microcentrifuge

Fig. 1: Stool Collection Tube with DNA Stabilizer

PROTOCOL

Note: In bold, modifications of the standard protocol are indicated.

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. **Vortex stabilized sample in Stool Collection Tube with DNA Stabilizer. Transfer 800 µl of this suspension (wide bore tips are recommended for pipetting the sticky material*)** and 800 µl of Solution CD1 to the PowerBead Pro Tube. Vortex briefly to mix
2. **Transfer the PowerBead Pro Tube in a bullet blender and homogenize for 2 min at full speed.**
3. Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.
4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).
   Note: Expect 500–600 µl. The supernatant may still contain some stool particles.
5. Add 200 µl of Solution CD2 and vortex for 5 s.
6. Centrifuge at 15,000 x g for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided). Note: Expect 500–600 µl.
7. **Add 300 µl of Solution CD3 and 300 µl 2-Propanol and vortex for 5 s.**
8. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x g for 1 min.
9. Discard the flow-through and repeat step 8 to ensure that all the lysate has passed through the MB Spin Column.
10. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.
11. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.
12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

* https://www.sigmaaldrich.com/
https://www.thermofisher.com/order/catalog/product/2079GPK

13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.
14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).
15. Centrifuge at up to 16,000 x g for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
16. Add 50–100 µl of Solution C6 to the center of the white filter membrane.
17. Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Note: store the DNA frozen (–30°C to –15°C or –90°C to –65°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide in the corresponding Qiagen Handbook.

This protocol was developed by Invitek and is for research use only, not for use in diagnostic procedures. Users are responsible for checking the suitability of the protocol for their application and adapting it if necessary.
For any questions please contact techsupport@invitek.com

RESULTS

To evaluate yield and quality of the isolated DNA, measurements with the Nanodrop spectral photometer (purity, yield) and TapeStation using Genomic DNA ScreenTape (integrity, yield) have been done. The modified protocol for isolation of 800 µl stabilized sample has been compared to the standard protocol for the QIAamp Power Fecal DNA Kit, where 250 mg of frozen sample were used as input sample material. It was shown that the modified protocol results in high yields and quality, superseding the standard protocol when using a combination of Invitek stabilization and Qiagen extraction, as displayed in Tab. 1 and Fig 2.

Tab. 1: DNA concentrations measured with Nanodrop and TapeStation. Orange (Q): Frozen samples isolated with the Qiagen standard protocol. Green (Mod): stabilized samples, isolated with the modified protocol.

Analysis	Nanodrop			TapeStation	
Sample	ng/µl	A 260:280	A 260:230	ng/µl	DIN
Q 1	46,22	2,01	0,19	19,4	6,2
Q 2	36,64	1,88	0,82	17,1	6,1
A2_1	367,09	2,07	1,55	48,5	7,3
A2_ 2	366,21	2,08	1,74	46,6	7,5

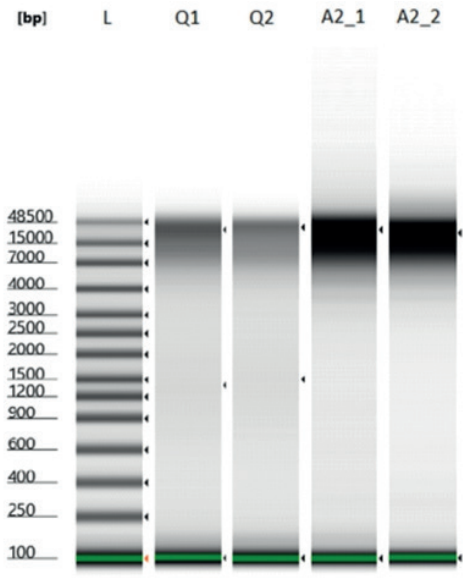
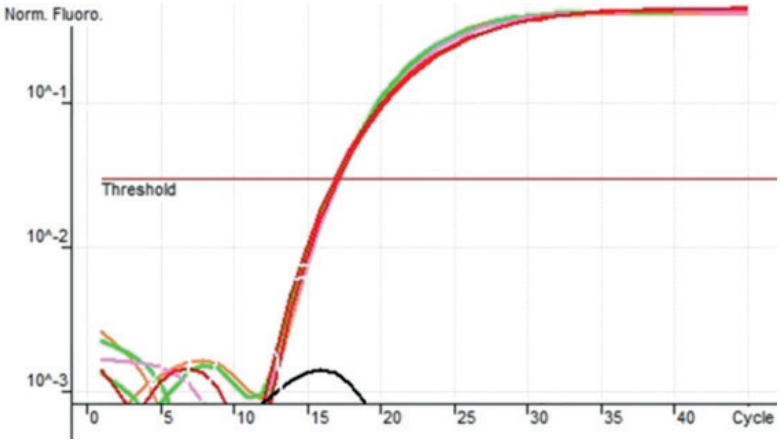


Fig. 2: TapeStation measurement, L: Ladder, Q1-Q2: frozen samples isolated with the standard protocol, A2_1-A2_2: stabilized samples isolated with the modified protocol

To test whether the protocol changes and addition of 2-propanol in step 7 of the isolation protocol affect the performance in downstream processes, real-time PCR was carried out using the InviQuant GeneCount 40 Kit (Invitek) according to the instructions for use. Standard human DNA (included in the InviQuant GeneCount 40 Kit) was mixed with sample eluates as PCR template. Reaction mixes consisted of 10 µl master mix, 2.5 µl sample eluate and 1 µl standard DNA. As a positive control, only the standard DNA was tested (PTC). As a second control, the elution buffer (Solution C6) was tested for any inhibitory effects. As shown in Fig. 3, there are no inhibitory effects on PCR. The results for standard and modified protocols are equivalent and correspond to the result of the positive control.



No.	Colour	Name	Ct
2	●	Q 1	17,22
3	●	Q 2	17,08
4	●	A2.1	17,07
5	●	A2.2	17,08
6	●	Elution buffer	17,32
7	●	PTC	17
8	●	PTC	17,22
9	●	NTC	

Fig. 3: Real-time PCR assay for samples isolated with the standard and the modified protocol. 2-3: samples isolated with the standard protocol, 4-5: samples isolated with the modified protocol, 6: elution buffer C6, 7-8: positive control, 9: negative control

CONCLUSIONS

- Human fecal samples collected with the Invitek Stool Collection Tubes with DNA Stabilizer can be successfully processed with the QIAamp Power Fecal Pro DNA Kit using the modified protocol outlined above.
- The modified protocol results in high yields and good DNA quality.
- RT-PCR demonstrates that the modification of the standard protocol does not impact downstream analysis. Isolated DNA is free of any inhibitors.

INVITEK DIAGNOSTICS GERMANY
Invitek Molecular GmbH
Robert-Roessle-Str. 10
13125 Berlin, Germany

Phone: +49 30 9489 2908

info@invitek.com

INVITEK DIAGNOSTICS PORTUGAL
ALS Life Sciences, SA
Zona Industrial de Tondela, ZIM II, Lote 6
3460-070 Tondela, Portugal

Phone: +351 232 817 817