Application Note

Purification of microbial DNA by combining Stool Collection Tubes with DNA Stabilizer (Invitek) and the ZymoBIOMICS™ DNA/RNA Miniprep Kit (Zymo Research)

The ZymoBIOMICS™ DNA/RNA Miniprep Kit is intended for the isolation of fresh or frozen stool samples, or samples stabilized with the DNA/RNA Shield™. 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: 750 µl stabilized sample

Extraction Kit: ZymoBIOMICS™ DNA/RNA Miniprep Kit (Cat. no. R2002, Zymo Research)

Reagent: 2-Propanol

Equipment: Microcentrifuge
Bullet Blender, or comparable bead mill homogenizer

Fig. 1: Stool Collection Tube with DNA Stabilizer

PROTOCOL

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

Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless otherwise specified.

1. **Vortex stabilized sample in Stool Collection Tube with DNA Stabilizer. Transfer 750 µl of this suspension (wide bore tips are recommended for pipetting*)** and 750 µl DNA/RNA Shield™ to a ZR Bashing Bead Lysis Tubes and cap tightly.
2. **Homogenize in Bullet blender (2 Min, Speed 9).**
3. Centrifuge the ZR BashingBead™ Lysis Tubes in a microcentrifuge for **1 minute**.
4. Transfer 400 µl supernatant to a new microcentrifuge tube (not provided).
5. **Add 400 µl of DNA/RNA Lysis Buffer and 400 µl of 2-Propanol to the sample and vortex for 10s.**
6. Transfer 600µl into a Spin-Away™ Filter (yellow) in a Collection Tube and centrifuge. Discard the flow-through. Transfer the next 600µl into a Spin-Away™ Filter (yellow) and centrifuge. Discard the flow-through.
7. Transfer the Spin-Away™ Filter (yellow) into a new Collection Tube.
8. Add 400 µl DNA/RNA Prep Buffer to the column and centrifuge. Discard the flow-through.
9. Add 700 µl DNA/RNA Wash Buffer to the column and centrifuge. Discard the flow-through.
10. Add 400 µl DNA/RNA Wash Buffer to the column and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into a nuclease-free tube (not provided).
11. Add 100 µl ZymoBIOMICS™ DNase/RNase-Free Water directly to the column matrix, incubate for 5 minutes, and then centrifuge to elute DNA from the respective column.

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

12. Alternatively, for highly concentrated DNA use ≥ 50 µl elution.
13. Inhibitor removal by Zymo-Spin™ III-HRC Filter: Place Zymo-Spin™ III-HRC Filter in a Collection Tube and add 600 µl ZymoBIOMICS™ HRC Prep Solution. Centrifuge at 8,000 x g for 3 minutes.
14. Transfer the eluted DNA into a prepared Zymo-Spin™ III-HRC Filter in a new microcentrifuge tube and centrifuge at 16,000 x g for 3 minutes. The flow through contains your DNA.

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 750 µl stabilized sample has been compared to the standard protocol of the ZymoBIOMICS™ DNA/RNA Miniprep Kit, without addition of 2-Propanol. It was shown that the modified protocol results in high yields and quality, superseding the standard protocol, as displayed in Tab. 1 and Fig 2.

Tab. 1: DNA concentrations measured with Nanodrop and TapeStation. Orange (St): stabilized samples isolated with the ZymoBIOMICS Standard protocol. Green (Md): stabilized samples, isolated with the modified protocol.

Analysis	Nanodrop			TapeStation	
Sample	ng/µl	A 260:280	A 260:230	ng/µl	DIN
St 1	15,12	2,16	0,91	11,9	6,1
St 2	17,63	2,07	0,93	15,5	6,1
Md 1	538,49	1,93	2,29	210	5,7
Md 2	511,22	1,93	2,27	171	5,6

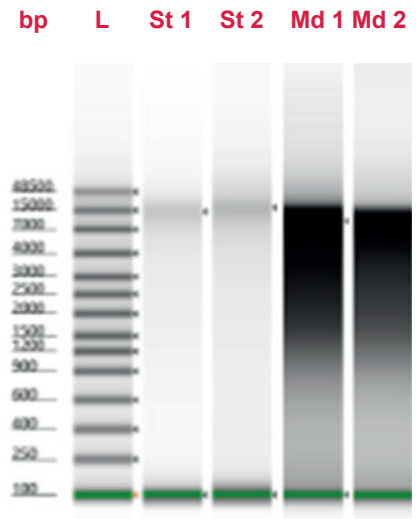
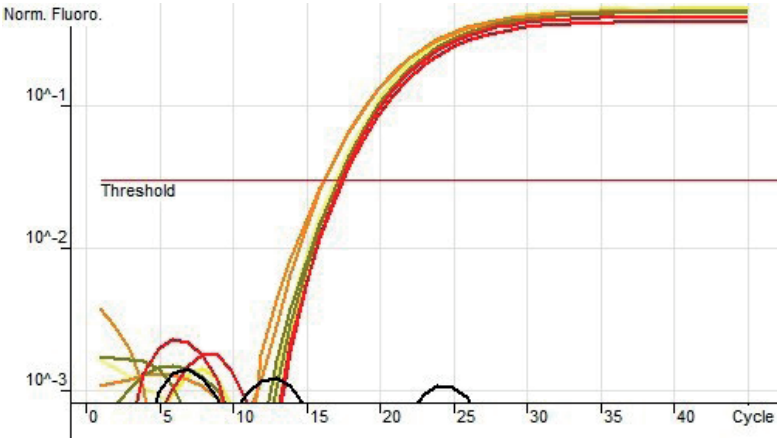


Fig. 2: TapeStation measurement, L: Ladder, St1/St2: stabilized samples isolated with standard protocol, Md1/Md2: stabilized samples isolated with modified protocol.

To test whether the protocol changes and addition of 2-propanol in step 5 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 (ZymoBIOMICS™ DNase/RNase-Free Water) was tested for any inhibitory effects. As shown in Fig. 3, there are no inhibitory effects on PCR. The DNA extracted with the modified protocol performs equally well for all samples and corresponds to the result of the positive control.



No.	Colour	Name	Ct
1		St 1	17,14
2		St 2	17,00
3		Md 1	16,33
4		Md 2	16,28
5		elution buffer	17,26
6		elution buffer	17,25
7		PTC	17,43
8		PTC	17,60
9		NTC	
10		NTC	

Fig. 3: Real-time-PCR assay for samples isolated with the modified protocol. 1-2: stabilized sample isolated with the standard protocol, 3-4: stabilized samples isolated with the modified protocol, 5-6: ZymoBIOMICS™ DNase/RNase-Free Water (elution buffer), 7-8: positive control (PTC), 9-10: negative control (NTC).

CONCLUSIONS

- Human fecal samples collected with the Invitek Stool Collection Tubes with DNA Stabilizer can be successfully processed with the ZymoBIOMICS™ DNA/RNA Miniprep Kit using the modified protocol outlined above.
- The modified protocol results in high yields and good DNA quality.
- Real-time 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