Application Note

Purification of microbial DNA by combining Stool Collection Tubes with DNA Stabilizer (Invitek) and the NucleoSpin® DNA Stool (Macherey-Nagel)

The NucleoSpin® DNA Stool 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: 850 µl stabilized sample

Extraction Kit: NucleoSpin® DNA Stool (REF 740472.XX, Macherey-Nagel)

Reagent: 2-Propanol

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

Fig. 1: Stool Collection Tube with DNA Stabilizer

PROTOCOL

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

1. Prepare sample

Vortex stabilized sample in Stool Collection Tube with DNA Stabilizer. Transfer 850 µl stabilized sample (wide bore tips are recommended for pipetting*) into MN Bead Tube. (Do NOT add Buffer ST1). Close the MN Bead Tube and shake horizontally for 2–3 seconds to mix stool sample and lysis buffer before putting it onto a heat incubator.

2. Lyse sample

Incubate MN Bead Tube for 5 min at 90 °C. Homogenize in Bullet blender (2 Min, Speed 9).

3. Precipitate contaminants

Centrifuge for 3 min at 13,000 x g. **(Do NOT add Buffer ST2).**

4. Filter lysate

Place a NucleoSpin® Inhibitor Removal Column (red ring) in a Collection Tube (2 ml, lid). Avoiding the pellet, transfer **400 µl** of the cleared lysate onto the NucleoSpin® Inhibitor Removal Column. Centrifuge for 1 min at 13,000 x g. Discard the NucleoSpin® Inhibitor Removal Column.

5. Adjust binding conditions

Add **200 µl 2-Propanol** and close the lid. Vortex for 5 s.

6. Bind DNA

Place a NucleoSpin® DNA Stool Column (green ring) in a Collection Tube (2 ml). Load **the whole sample** onto the column. Centrifuge for 1 min at 13,000 x g. Discard flowthrough and place the column back into the collection tube.

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

7. Wash silica membrane

1st wash: This step is skipped!

2nd wash: Add 550 µl Buffer ST4 to the NucleoSpin® DNA Stool Column. Centrifuge for 1 min at 13,000 x g. Discard flowthrough and place the column back into the collection tube.

3rd wash: Add 700 µl Buffer ST5 to the NucleoSpin® DNA Stool Column. Close the lid and vortex for 2 s. Centrifuge for 1 min at 13,000 x g. Discard flowthrough and place the column back into the collection tube.

4th wash: Add 700 µl Buffer ST5 to the NucleoSpin® DNA Stool Column. Centrifuge for 1 min at 13,000 x g. Discard flowthrough and place the column back into the collection tube.

8. Dry silica membrane

Centrifuge for 2 min at 13,000 x g.

9. Elute DNA

Place the NucleoSpin® DNA Stool Column into a new 1.5 ml microcentrifuge tube (not provided). Add 30 µl (for high concentration), 50 µl (for medium concentration and yield), or 100 µl (for high yield) Buffer SE to the column. Close the lid and centrifuge for 1 min at 13,000 x g. Discard the NucleoSpin® DNA Stool Column. Vortex each microcentrifuge tube for 2 s.

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 850 µl stabilized sample has been compared to the standard protocol of the NucleoSpin® DNA Stool Kit, without addition of 2-Propanol. With the standard protocol, the yield was very low and below the detection level for the TapeStation measurement. To check for reproducibility of the modified protocol, stabilized samples that had been frozen at -80°C have been isolated as well. It was shown that the modified protocol results in high yields and quality for all stabilized samples, 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 standard protocol. Green (Md): stabilized samples, isolated with the modified protocol. Grey (-80): stabilized samples, that have been frozen at -80°C, isolated with the modified protocol.

Analysis	Nanodrop			TapeStation	
Sample	ng/µl	A 260:280	A 260:230	ng/µl	DIN
St 1	2,21	1,12	0,15	-	-
St 2	2,48	2,78	0,1	-	-
Md 1	387,21	2	2,16	106	6,6
Md 2	343,01	2	2,09	109	6,6
-80 1	409,20	2,03	2,33	96,14	7,5
-80 2	368,91	2,07	2,33	76,8	7,3

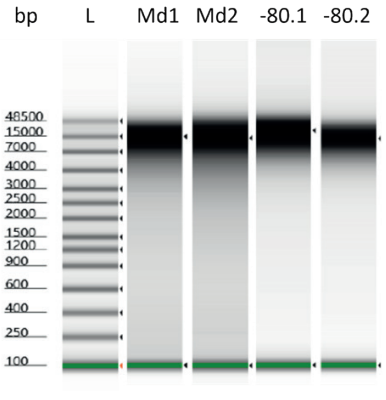
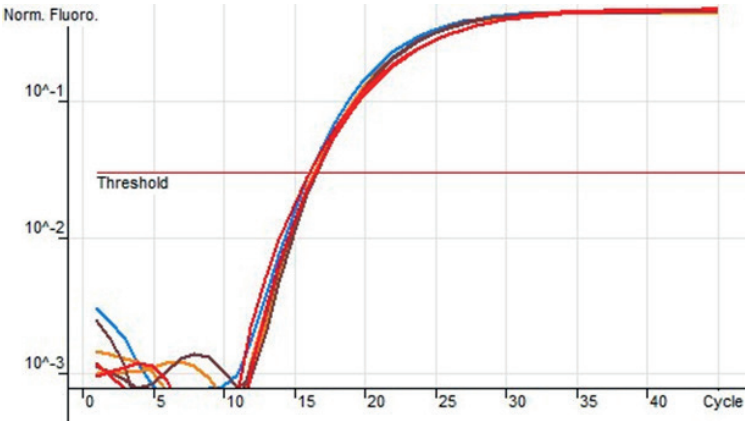


Fig. 2: TapeStation measurement, L: Ladder, Md 1 / Md 2: stabilized samples isolated with standard protocol, -80.1 / -80.2: stabilized samples, that have been frozen at -80C, isolated with modified protocol.

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 (Buffer SE) 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		Md 1	16,21
2		-80 1	16,46
3		-80 2	16,52
4		Buffer SE	16,59
5		PTC	16,18
6		PTC	16,58
7		NTC	
8		NTC	

Fig. 3: Real-time-PCR assay for samples isolated with the modified protocol. 1: stabilized sample isolated with the modified protocol, 2-3: stabilized samples, that have been frozen at -80°C, isolated with the modified protocol, 4: Buffer SE (elution buffer), 5-6: positive control (PTC), 7-8: negative control (NTC)

CONCLUSIONS

- Human fecal samples collected with the Invitek Stool Collection Tubes with DNA Stabilizer can be successfully processed with the NucleoSpin® DNA Stool kit from Macherey-Nagel 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.

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