SphaeraMag® Genomic DNA Fecal Purification Kit

High yield of pure DNA with good integrity for microbiome or metagenome analyses

LABORATORY USE ONLY

INSTRUCTION MANUAL VERSION 1.5

Procomcure Biotech (PCC)

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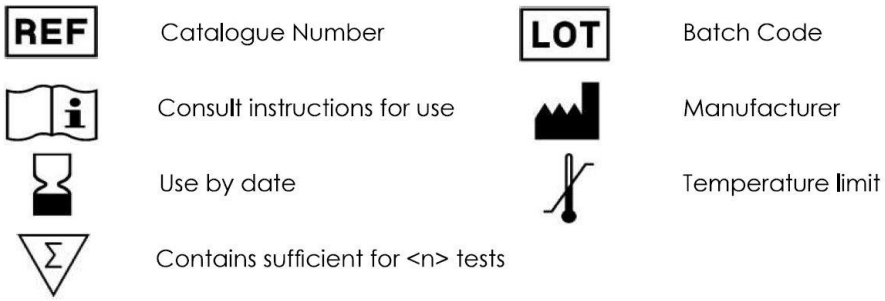
Explanation of Symbols



1. Product highlights

- Validated, unbiased protocol for microbiome analysis.
- Bead bashing guarantees complete homogenization and disruption of microbial cells.
- Simple workflow: bead-bash sample, purify and elute.
- Flexible workflow: available for manual and automated platforms.

2. Specifications

Sample source: Bacterial (Gram-negative/positive), fungal, plant, viral and host DNA is efficiently isolated from ~100 mg feces.

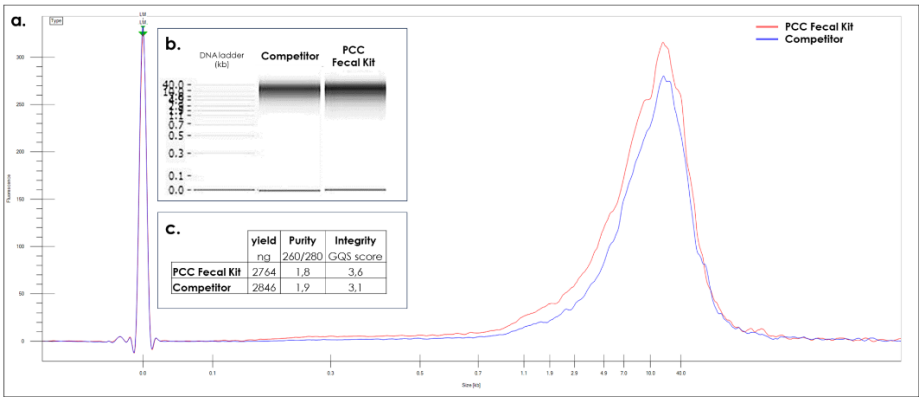
Bead bashing system: Procomcure's (PCC's) bead bashing system enables complete homogenization/disruption of the microbial cell walls and accurate, bias-free microbial DNA analysis.

DNA yield (Fig. 1c): The expected yield is up to ~3 µg DNA from ~100 mg feces.

DNA purity (Fig. 1c): High quality DNA can be obtained.

DNA integrity (Fig. 1a-c): Depending on the quality of the input sample, on average the size of the extracted DNA is between 15-20 kb and the average Genomic DNA Quality Score (GQS) is 3,6. GQS represents the degree of degradation of a sample with 5 corresponding to intact DNA and 0 corresponding to highly degraded DNA.

Bioburden: A single preparation is guaranteed to contain less than 3 bacterial genomic copies per µl of eluate as determined by quantitative amplification of the 16S rRNA gene when eluted using 50 µl water.



	yield	Purity	Integrity
	ng	260/280	GQS score
PCC Fecal Kit	2764	1,8	3,6
Competitor	2846	1,9	3,1

Figure 1. Quantitative and qualitative properties of the DNA extracted with SphaeraMag® Genomic DNA Fecal Purification Kit vs a competitor kit: **a.**, DNA integrity was assessed with the Genomic DNA Assay by PerkinElmer run on a LabChip GX device. The electropherogram shows genomic DNA isolated with the SphaeraMag® Genomic DNA Fecal Purification Kit (orange) vs a competitor kit (blue), **b.**, the gel photo illustrates that high molecular weight intact DNA can be isolated with the SphaeraMag® Genomic DNA Fecal Purification Kit. **c.**, Numerical values showing the yield, purity and integrity properties of DNA isolated with the SphaeraMag® Genomic DNA Fecal Purification Kit versus a competitor kit.

2.1. Downstream applications

The SphaeraMag® Genomic DNA Fecal Purification Kit generates excellent input material for sensitive downstream applications such as quantitative PCR (qPCR) (Fig. 2a) or library preparation for Next Generation Sequencing (NGS) purposes (Fig. 2b).

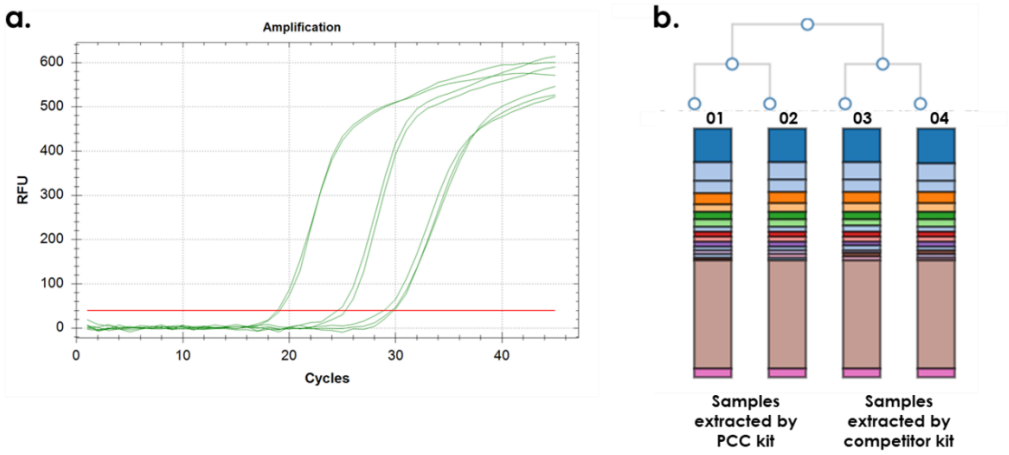


Figure 2. Inhibitor and bias-free microbial DNA extraction using SphaeraMag® Genomic DNA Fecal Purification Kit from human stool: **a.**, DNA was extracted from human stool sample and was diluted to produce a 10-log range of template concentrations. The amplification plot shows the result of the qPCR obtained using the PhoenixDx® Pan Bacteria (PCCSKU15329) assay which targets bacterial-specific genomic sequence. Samples were run on a Real Time PCR Cycler (CFX-96) from Bio-Rad and the plot was generated with the Bio-Rad CFX Maestro software. **b.**, DNA from a commercially available human stool standard was extracted either with the SphaeraMag® Genomic DNA Fecal Purification Kit or with a competitor kit. Then NGS libraries were prepared from the extracted DNA by an amplicon-based 16S approach. The libraries were sequenced in a NextSeq2000 sequencing system. The sequencing data was analyzed by the Metagenomics Lab application (Illumina). The dendrogram shows hierarchical clustering of samples based on genus-level classifications. The bar chart shows the relative, genus-level abundances.

3. Kit components

	SphaeraMag® Fecal DNA Kit – Universal 96	**SphaeraMag® Fecal DNA Kit – Pre-filled 96**	**SphaeraMag® Fecal DNA Kit – Pre-filled 32**	**SphaeraMag® Fecal DNA Kit – Pre-filled Mini**
Catalogue No.	**PCCSKU16098**	**PCCSKU16099**	**PCCSKU16100**	**PCCSKU16101**
No. of Preps	96	96	80	48
No. of bead-beating tubes	96	96	80	48
Enhancer Solution	4 ml	Pre-filled	Pre-filled	Pre-filled
Lysis/Binding Buffer	20 ml	20 ml	20 ml	10 ml
Wash Buffer I	47 ml	Pre-filled	Pre-filled	Pre-filled
Wash Buffer II	27 ml	Pre-filled	Pre-filled	Pre-filled
Elution Buffer	10 ml	Pre-filled	Pre-filled	Pre-filled
Proteinase K	2 x 1 ml	2 x 1ml	2 x 1ml	1 ml
Magnetic Beads	4 x 1 ml	4 x 1 ml	4 x 1 ml	2 x 1 ml
Tip comb(s)	-	1 x 96 tip comb (in holder)	10 x 8 tip combs	30 x 8 tips combs

Table 1. The SphaeraMag® Genomic DNA Fecal Purification Kit content.

4. Product description

4.1. Introduction

The SphaeraMag® Genomic DNA Fecal Purification Kit is designed for rapid and reliable isolation of DNA from fecal samples. Samples are bead bashed and lysed in carefully formulated buffers to release nucleic acids. The isolated DNA is bound to the surface of paramagnetic beads, while proteins and cellular debris are removed during washing steps. The DNA is then eluted in the provided Elution Buffer. Alternatively, nuclease-free water or other low ionic strength buffers can be used. The procedure can be performed using automated systems (like Phoenix-Pure) or manually. The purified DNA is suitable for several downstream applications such as qPCR, end-point PCR, SNP analysis, genotyping, microarray analysis and amplicon based (16S, ITS) or shotgun library preparation for next generation sequencing (NGS).

4.2. Kit compatibility

	Universal 96	**Pre-filled 96**	**Pre-filled 32**	**Pre-filled Mini**
Phoenix-Pure 96/Auto-Pure 96	+	+		
Phoenix-Pure 32/Auto-Pure 32	+		+	
Auto-Pure Mini	+			+
KingFisher™ Flex Purification System	+	+		

Table 2. SphaeraMag® Genomic DNA Fecal Purification Kit compatibility.

4.3. Storage and stability

Proteinase K enzyme must be stored at -25 to -15 ℃, Magnetic Beads at +2 to +8 ℃ upon arrival. All other kit components are stored at room temperature (+15 to +25 ℃). The expiry dates of the kit and single components are stated on the outer packaging as well as on the individual reagents.

4.4. Precaution

Proper laboratory safety practices should be employed, including the use of lab coat, gloves and protective goggles. For information regarding the composition of buffers, please check the Safety Data Sheets (available upon request). Each reagent is optimized for the specific use with this kit. Do not substitute reagents from any other manufacturer into the kit and do not combine with different LOT numbers.

4.5. Procedure overview

Fecal samples are first bead bashed, then treated with Proteinase K in combination with Enhancer Solution. In the following step, a specifically formulated chaotropic salt-based Lysis/Binding Buffer is used to simultaneously lyse cells, degrade protein components, and bind the extracted DNA to the surface of paramagnetic beads. Additionally, the buffer offers protection against high nuclease activity. A three-step washing procedure, including 2 washing buffers, is performed to remove proteins, detergents, and other impurities. The extracted DNA is then released from the magnetic beads using Elution Buffer.

4.6. Required materials - not supplied

General

- Ethanol, absolute (only for Universal 96)
- Isopropanol ≥99.5% (only for Universal 96)
- Suitable reagent and sample dispensing options
- Consumables for isolation devices for automated extraction
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)
- Bead bashing device
- Vortexer

Specific for manual purification

- Thermal mixer for 1.5 ml or 2 ml tubes (or alternative heating device)
- Water bath (or alternative heating device to pre-heat buffers)
- Magnetic separation rack for 1.5 ml or 2 ml tubes

4.7. Sample storage and handling guidelines

Appropriate storage of sample material is essential to avoid the introduction of post-collection bias in microbial community composition. To ensure a high-quality DNA, fast processing (within 48 hours) of samples is highly recommended. If this is not possible either rapid freezing to −20 to -80 °C or storage in Phoenix Protect (#PCCSKU16009, Procomcure Biotech) or similar inactivation/conservation solution for up to 72 hours is recommended.

4.8. Pre-application preparation

Make sure to get familiar with the complete extraction process before getting started.

GENERAL IMPORTANT NOTES BEFORE GETTING STARTED

- Prepare Wash Buffer I (only for Universal 96) by adding Isopropanol ≥99.5% (not supplied) as indicated on the bottle label and store at room temperature.
- Prepare Wash Buffer II (only for Universal 96) by adding Ethanol, absolute (not supplied) as indicated on the bottle label and store at room temperature.
- When using an alternative to a thermal mixer (*e.g.* heating block, incubator or water bath), vortex sample at a repetitive frequency for approx. 5 min (depending on the overall incubation time).
- Lysis/Binding Buffer, Wash Buffer I and Enhancer Solution may form precipitates when stored under cool conditions. Check individually bottled buffers for precipitation before use. Re-dissolve at +37°C and gently mix, if necessary.

IMPORTANT NOTES ON ELUTION BUFFER AND THE STORAGE OF THE EXTRACTED DNA

- The Elution Buffer is 10 mM Tris-HCl, pH 8.0. Alternatively, nuclease-free water or other low ionic strength buffers can be used.
- Elution is strongly recommended with the Elution Buffer pre-heated to +60°C to improve elution efficiency.
- The recommended elution volume ranges between 50-100 µl. A higher elution volume results in higher yield, but decreased DNA concentration. A lower volume allows for higher concentration.
- The eluted DNA can be safely stored at +4 to +8°C for weeks to months. For long term storage, store the eluted DNA at -80°C. Repeated freeze-thaw cycles should be avoided to maintain eluate integrity.

5. Extraction protocols

5.1. Bead bashing procedure

- Add fecal samples (~100 mg solid or ~200 µl liquid) to 2 ml screw cap tube(s) pre-filled with bashing beads.
- Add 750 µl Phoenix Protect (or similar inactivation/conservation solution) to each processed tube/sample.
- Samples can be bead bashed in either a bead bashing device like the Bead Ruptor 96 * (Omni International) at 12 Hz for 5 minutes or with a vortex mixer equipped with a vertical microtube holder at maximum speed for 40 minutes.
- Centrifuge the tubes at 3,900x g for 5 min in a bench top centrifuge.
- As an input, use 200 µl supernatant in the automated/manual extraction protocols.

* The exact conditions dependent on the used mechanical device must be empirically validated to ensure unbiased lysis.

5.2. Automated protocols

5.2.1. Automated purification: Auto-Pure 96/Phoenix-Pure 96

Compatible Kits: SphaeraMag® Genomic DNA Fecal Purification Kit – Universal 96

SphaeraMag® Genomic DNA Fecal Purification Kit – Pre-filled 96

Important notes:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled plates are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. If necessary, centrifuge the deep-well plate(s) at 2,000x g for 3 minutes to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Make sure to have V-bottom 96 deep-well plates containing reagents (Table 3) ready for use.

Step Name	Plate Position	Reagents	Volume per well
Loading	1	Tip comb	-
Digestion	2	Enhancer Solution	40 µl
-	3	-	-
Wash 1	4	Wash Buffer I	600 µl
Wash 2	5	Wash Buffer II	600 µl
Wash 3	6	Wash Buffer II	600 µl
-	7	-	-
Elution	8	Elution Buffer	50 µl

Table 3. Setup for V-bottom 96 deep-well plates compatible with Phoenix-Pure 96/Auto-Pure 96 device.

2. Remove sealing foil from pre-filled plates.

3. Transfer sample(s) and Proteinase K (orange) to appropriate well(s) (Table 4) on Position-2 Plate.

   Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

Step Name	Plate Position	Sample/Reagents	Volume per well
Loading	1	Tip comb	-
Digestion	2	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
-	3	-	-
Wash 1	4	Wash Buffer I	600 µl
Wash 2	5	Wash Buffer II	600 µl
Wash 3	6	Wash Buffer II	600 µl
-	7	-	-
Elution	8	Elution Buffer	50 µl

Table 4. Pipetting scheme for sample and Proteinase K transfer into V-bottom 96 deep-well plate prior to program 1 of 2.

4. Load V-bottom 96 deep-well plates onto instrument's plate positions according to plate layout (Table 4). Note: Make sure that all plates are placed in the correct orientation.

5. Set program 1 of 2 according to Table 5.

Step	Step name	plate	Mix time (min)	Mix amp (%)	Wait time (min)	Volume (µl)	Mix speed (1-10)	Temp (°C)	Segment (1-5)	Cycle time (1-10)	Magnet speed (1-10)	1st segment time	2nd segment time
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Digest	2	20	80	0	300	3	65	2	1	3	15	15
3	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 5. Program setting for step 1 of 2 for the Phoenix-Pure 96/Auto-Pure 96 device.

6. Run the program.

7. Once the program is finished, take the Position-2 Plate out of the device.

8. Mix the Lysis/Binding Buffer with Magnetic Beads in a nuclease-free tube for required amount of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads.

Note: Ensure that beads stay fully mixed by constantly inverting the tube.

9. Transfer 240 µl Lysis/Binding Buffer and Magnetic Bead mix to each used well on the Position-2 Plate.

10. Load Position-2 Plate back onto the device.

11. Set program 2 of 2 according to Table 6.

Step	Step name	plate	Mix time (min)	Mix amp (%)	Wait time (min)	Volume (µl)	Mix speed (1-10)	Temp (°C)	Segment (1-5)	Cycle time (1-10)	Magnet speed (1-10)	1st segment time	2nd segment time
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Lysis/Bind	2	5	80	0	500	3	37	2	1	4	20	20
3	Wash 1	4	1	80	0	600	5	0	2	1	3	20	20
4	Wash 2	5	1	80	0	600	5	0	2	1	3	20	20
5	Wash 3	6	2	80	2.5	600	5	0	2	1	3	20	20
6	Elution	8	1	80	0	100	3	60	2	2	3	30	30
7	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 6. Program setting for step 2 of 2 for the Phoenix-Pure 96/Auto-Pure 96 device.

12. Run the program.

13. After the run is finished, remove V-bottom 96 deep-well plates and the tip comb. Transfer eluates from Position-8 plate (Elution Buffer) into new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

5.2.2. Automated purification: Auto-Pure 32/Phoenix-Pure 32

Compatible Kits: SphaeraMag® Genomic DNA Fecal Purification Kit - Universal 96

SphaeraMag® Genomic DNA Fecal Purification Kit - Pre-filled 32

Important notes:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled plates are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. If necessary, centrifuge the deep-well plate(s) at 2,000x g for 3 minutes to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Make sure to have U-bottom 96 deep-well plate(s) containing reagents (Table 7) ready to use.

Well	Reagents	Volume per well
Column 1/7	Enhancer Solution	40 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	50 µl

Table 7. Setup for an U-bottom 96 deep-well plate compatible with Phoenix-Pure 32/Auto-Pure 32 device.

2. Remove sealing foil from pre-filled plate(s).

3. Transfer sample(s) and Proteinase K (orange) to appropriate well(s) in columns 1/7 (Table 8).

Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

Well	Sample/Reagents	Volume per well
Column 1/7	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	50 µl

Table 8. Pipetting scheme for sample and Proteinase K transfer into U-bottom 96 deep-well plate(s) prior to program 1 of 2.

4. Load U-bottom 96 deep-well plate(s) onto the instrument. Note: Make sure that each plate is placed in the correct orientation.

5. Place new clean tip comb(s) in the instrument.

6. Set program 1 of 2 according to Table 9.

Step	Step name	well	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µl)	Mix speed (1-10)	Temp (°C)	Mix pos (%)	Mix amp (%)	Magnet pos (%)	Magnet speed (1-10)
1	Digest	1	20	68	0	300	7	65	0	80	0	3

Table 9. Program setting for step 1 of 2 for the Phoenix-Pure 32/Auto-Pure 32 device.

7. Run the program.

8. Once the run is finished, leave tip comb(s) in the device and take the U-bottom 96 deep-well plate(s) out of the device.

9. Mix the Lysis/Binding Buffer with Magnetic Beads in a nuclease-free tube for required number of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads.

Note: Ensure that beads stay fully mixed by constantly inverting the tube.

10. Transfer 240 µl Lysis/Binding Buffer and Magnetic Bead mix to appropriate wells in column(s) 1/7.

11. Load the U-bottom 96 deep-well plate(s) back onto the device.

12. Set program 2 of 2 according to Table 10.

Step	Step name	well	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µl)	Mix speed (1-10)	Temp (°C)	Mix pos (%)	Mix amplitude (%)	Magnet pos (%)	Magnet speed (1-10)
1	Lysis/Bind	1	5	68	0	500	4	37	0	80	0	4
2	Wash 1	3	1	68	0	600	5	0	0	80	0	3
3	Wash 2	4	1	68	0	600	5	0	0	80	0	3
4	Wash 3	5	2	68	2.5	600	5	0	0	80	0	3
5	Elution	6	1	180	0	100	6	60	0	80	0	3
6	Waste	1	0.1	0	0	500	7	0	0	80	0	3

Table 10. Program setting for step 2 of 2 for the Phoenix-Pure 32/Auto-Pure 32 device.

13. Run the program.

14. After the run is finished, remove the U-bottom 96 deep-well plate(s) and tip comb(s). Transfer the eluate(s) from the column(s) 6/12 to new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

5.2.3. Automated purification: Auto-Pure Mini

Compatible Kit: SphaeraMag® Genomic DNA Fecal Purification Kit - Universal 96

SphaeraMag® Genomic DNA Fecal Purification Kit - Pre-filled Mini

Important Notes:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled cartridges are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. When using a V-bottom 96 deep-well plate, centrifuge the deep-well plate at 2,000x g for 3 minutes to remove droplets from the seal, if necessary.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

Samples can be processed (a) in cartridges (1 cartridge per sample) or (b) in a V-bottom 96 deep-well plate (16 samples per plate). Make sure to have reagents dispensed according to Table 11 or Table 12.

(a) Cartridge format:

Well	Reagents	Volume per well
Position 1	Enhancer Solution	40 µl
Position 2	-	-
Position 3	Wash Buffer I	600 µl
Position 4	Wash Buffer II	600 µl
Position 5	Wash Buffer II	600 µl
Position 6	Elution Buffer	50 µl

Table 11. Setup for a cartridge compatible with Auto-Pure Mini.

(b) V-bottom 96 deep-well format:

Well	Reagents	Volume per well
Column 1/7	Enhancer Solution	40 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	50 µl

Table 12. Setup for a V-bottom 96 deep-well plate compatible with Auto-Pure Mini.

1. Remove sealing foil from cartridge(s) or V-bottom 96 deep-well plate.

2. Transfer sample(s) and Proteinase K (orange) to appropriate well(s) according to Table 13 or Table 14.

Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

(a) Cartridge format:

Well	Reagents	Volume per well
Position 1	Enhancer Solution **Sample** **Proteinase K**	40µl **200 µl** **20 µl**
Position 2	-	-
Position 3	Wash Buffer I	600 µl
Position 4	Wash Buffer II	600 µl
Position 5	Wash Buffer II	600 µl
Position 6	Elution Buffer	50 µl

Table 13. Pipetting scheme for sample and Proteinase K transfer into cartridge(s) prior to program 1 of 2

(b) V-bottom 96 deep-well format:

Well	Reagents	Volume per well
Column 1/7	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	50 µl

Table 14. Pipetting scheme for sample and Proteinase K transfer into a V-bottom 96 deep-well plate prior to program 1 of 2.

3. Load cartridge(s) or V-bottom 96 deep-well plate onto instrument.
Note: Make sure that cartridge(s) or V-bottom 96 deep-well plate are placed in the correct orientation.

4. Place new clean tip comb(s) in the instrument.

5. Set program according to Table 15 or scan the protocol QR code to the right:

Fecalmini1-Protocol code-1/1

order	Step name	Hole site	Volume (µl)	Mix time (min)	Mix speed	Dry time (min)	Temperature (℃)	Segments	Every time (sec)	Magnetization time (sec)	Cycle	Magnet speed (mm/sec)	Total time (sec)	Mix scope (%)	Mix pos. (%)	Magnet pos. (%)
1	Digest	1	700	20	8	0	65	2	30	0	1	3	68	80	0	0
2	Waste	1	500	0.1	5	/	/	1	0	0	1	2.5	0	80	0	0

Table 15. Program setting for step 1 of 2 for Auto-Pure Mini device.

6. Run the program.

7. Once the run is finished, leave tip comb(s) in the device and take the cartridge(s) or the V-bottom 96 deep-well plate out of the device.

8. Mix the Lysis/Binding Buffer with Magnetic Beads in a nuclease-free tube for required number of reactions. For each sample: Mix 200 pl Lysis/Binding Buffer + 40 pl Magnetic Beads.

Note: Ensure that beads stay fully mixed by constantly inverting the tube.

9. Transfer 240 µl Lysis/Binding Buffer and Magnetic Bead mix to appropriate wells:

(a) Cartridge format: Position 1

(b) V-bottom 96 deep-well plate format: Column 1/7

10. Load cartridge(s) or the V-bottom 96 deep-well plate back onto the device.

11. Set program 2 of 2 according to Table 16 or scan the protocol QR code to the right:

Fecalmini2-Protocol code-1/1

order	Step name	Hole site	Volume (µl)	Mix time (min)	Mix Speed	Dry time (min)	Temperature (℃)	Segments	Every time (sec)	Magnetization time (sec)	Cycle	Magnet speed (mm/sec)	Total time (sec)	Mix scope (%)	Mix pos. (%)	Mix pos (%)	Magnet pos (%)
1	Bind	1	700	5	7	0	37	2	30	0	1	3	68	80	0	0	0
2	Wash 1	3	500	1	5	0	/	2	30	0	1	3	65	80	0	0	0
3	Wash 2	4	500	1	5	0	/	2	30	0	1	3	65	80	0	0	0
4	Wash 3	5	500	2	5	2.5	/	2	30	0	1	3	65	80	0	0	0
5	Elute	6	100	1	4	0	60	2	45	0	1	3	91	80	0	0	0
6	recyc	1	500	0.1	5	/	/	1	0	0	1	2.5	0	80	0	0	0

Table 16. Program setting for step 2 of 2 for Auto-Pure Mini device.

12. Run the program.

13. After the run is finished, remove cartridge(s) or the V-bottom 96 deep-well plate and tip comb(s). Transfer the eluate from position 6 or column(s) 6/12 to new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

5.3. Manual extraction

Graphical abstract

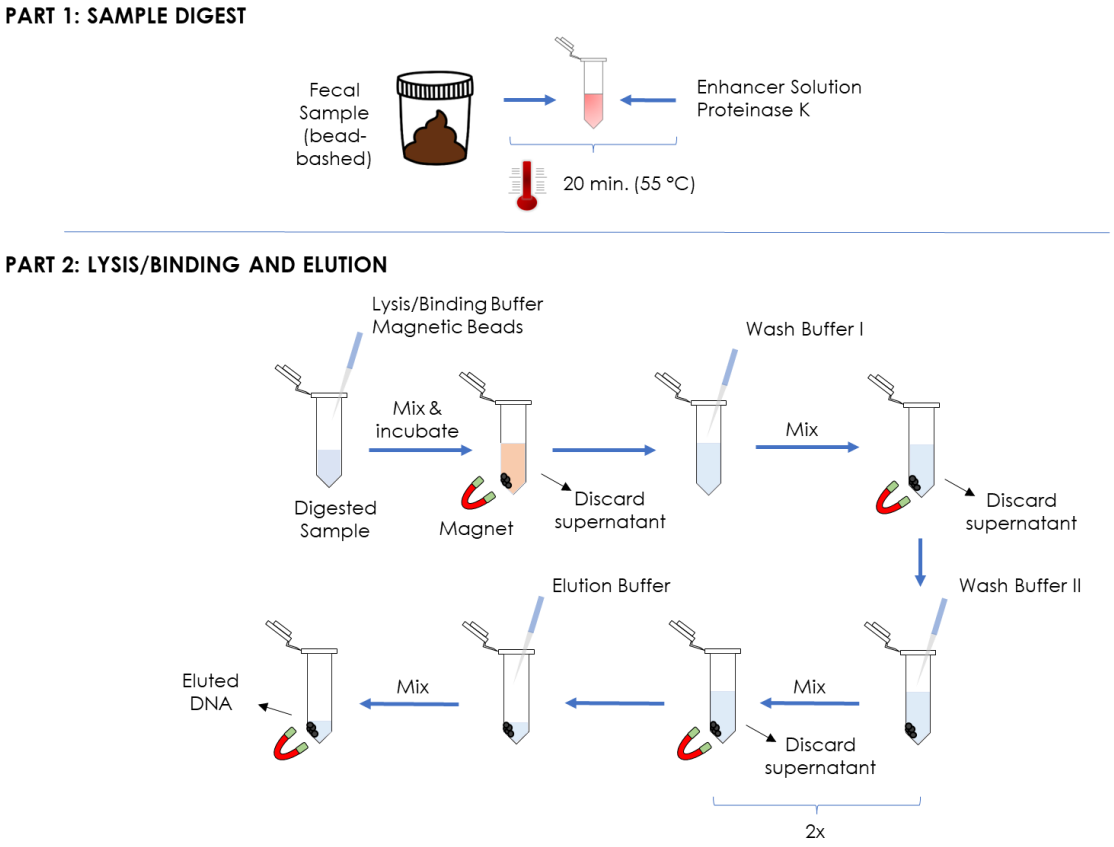


Figure 3. Graphical abstract of the manual workflow.

Preparation:

1. Bead bashing procedure.
    - Add fecal samples (~100 mg solid or ~200 µl liquid) to 2 ml screw cap tube(s) pre-filled with bashing beads.
    - Add 750 µl Phoenix Protect (or similar inactivation/conservation solution) to each processed tube/sample.
    - Samples can be bead bashed in either a bead bashing device like the Bead Ruptor 96 * (Omni International) at 12 Hz for 5 minutes or with a vortex mixer equipped with a vertical microtube holder at maximum speed for 40 minutes.
    - Centrifuge the tubes at 3,900x g for 5 min in a bench top centrifuge.
    - As an input, use 200 µl supernatant in the automated/manual extraction protocols.

    * The exact conditions dependent on the used mechanical device must be empirically validated to ensure unbiased lysis.

2. Pre-heat appropriate volume of Elution Buffer to +60°C.
3. Transfer 40 µl Enhancer Solution to a nuclease-free 1.5 ml or 2 ml microcentrifuge tube.
4. Add 200 µl supernatant of the bead bashed sample and add 20 µl Proteinase K to the tube. Mix by pipetting up and down at least 5 times.
5. Incubate in a thermal mixer at +55°C with agitation at full speed (~1,400 rpm) for 20 min.

    Alternative: Incubate in a heating device at +55°C for 20 min. Invert the tube ~5 times during incubation.

6. Vortex Magnetic Beads thoroughly. Combine them with the Lysis/Binding Buffer in a nuclease-free tube for required number of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads. Note: Ensure that beads stay fully mixed by constantly inverting the tube.
7. Pipette 240 µl Lysis/Binding Buffer and Magnetic Bead mix to the processed sample.
8. Incubate in a thermal mixer at +37°C with agitation at full speed (~1,400 rpm) for 5 min.

   Alternative: Incubate in a heating device at +37°C for 5 min. Invert the tube 2 times during incubation.

9. Place the tube on a magnetic stand. Allow the magnetic beads to settle until the solution is completely clear.
10. Remove and discard the supernatant. Avoid disturbing the magnetic bead pellet.
11. Add 600 µl Wash Buffer I.
12. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
13. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
14. Remove and discard the supernatant. Avoid disturbing magnetic bead pellet.
15. Add 600 µl Wash Buffer II.
16. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
17. Place the tube on the magnetic stand. Allow the magnetic beads to settle until the solution is completely clear.
18. Remove and discard the supernatant. Avoid disturbing the magnetic bead pellet.
19. Repeat steps (15-18) for an additional washing step with Wash Buffer II.
20. Remove any residual liquid. Leave the tube open for 5 min to allow the magnetic beads to air dry.
21. Add 50 µl pre-heated Elution Buffer to the tube.
22. Remove the tube from the magnetic stand. Mix well by pipetting up and down ~10 times.
23. Incubate at room temperature for 5 min.
24. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
25. Transfer the eluate to a clean tube. Process directly, or store at +4 to +8°C for short-term or at -80°C for long term storage.

26. Troubleshooting guide

Please use this guide for troubleshooting the possible problems that may arise. For further assistance, please contact the technical support staff at sales@procomcure.com.

Problem	Possible Cause	Solution
Low DNA Yield	Incomplete resuspension of SphaeraMag® Magnetic Beads	Thoroughly resuspend SphaeraMag® Magnetic Beads before use.
	DNA degradation during sample handling or storage	Process sample immediately after collection or removal from adequate storage.
	Incorrect preparation of Wash Buffers	Prepare Wash Buffers by adding the correct amount of ethanol or isopropanol according to instructions indicated on the bottle labels.
	Insufficient sample material	Increase lysis and binding time if the sample is diluted.
Problems with downstream applications	Poor DNA quality	Do not freeze-thaw the isolated gDNA more than once or store at room temperature.
	Insufficient DNA was used	Quantify the isolated gDNA accurately and use sufficient gDNA.
	Ethanol carry-over	Dry the SphaeraMag® Magnetic Beads completely before adding Elution buffer.
Carry-over of Magnetic Beads	Incomplete magnetization	Place the eluted samples on a magnetic stand device for additional 5 minutes or centrifuge at >4,000x g for 5 minutes. When using automated isolation, check the instrument settings and increase bead binding time, if necessary.

Table 17. Troubleshooting guide.

27. Consumables, related products and PCCs microbiome services

Product	Content	Cat.No.
SphaeraMag® Fecal DNA Kit – Universal 96	96 preps	PCCSKU16098
SphaeraMag® Fecal DNA Kit – Pre-filled 96	96 preps for Phoenix-Pure 96 & Auto-Pure 96	PCCSKU16099
SphaeraMag® Fecal DNA Kit – Pre-filled 32	80 preps for Phoenix-Pure 32 & Auto-Pure 32	PCCSKU16100
SphaeraMag® Fecal DNA Kit – Pre-filled Mini	48 preps for Auto-Pure Mini	PCCSKU16101
Tip Combs for Auto-Pure 96 & Phoenix-Pure 96	2 pcs	PCCSKU30014SA
Tip Combs for Auto-Pure 32, Phoenix-Pure 32 & Auto-Pure Mini	20 pcs	PCCSKU16013
U-Bottom Deep-well Plates for Auto-Pure 32 & Phoenix-Pure 32	20 pcs	PCCSKU16014
V-Bottom Deep-well Plates for Auto-Pure 96, Phoenix-Pure 96 & Auto-Pure Mini	50 pcs	PCCSKU30002
PhoenixProtect Sample Collection Kit	150 tubes	PCCSKU16062
PhoenixDx® Pan Bacteria	96 rxn	PCCSKU15329
PhoenixDx® Pan Fungi	96 rxn	PCCSKU15330

Table 18. Consumables, related products

Please check out our NGS Microbiome Services at
https://ngs.procomcure.com/microbiome/

We offer Targeted Sequencing Services (16S and ITS), shotgun metagenome analysis, with or without DNA extraction as well as with or without comprehensive bioinformatics analysis.

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"We make it real!"

Ordering information

For ordering **PCC-AMP 16S Panel Kit** and other products, visit us at

www.procomcure.com

or order via E-mail:

sales@procomcure.com

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