SphaeraMag® Genomic DNA Milk Purification Kit

Nucleic Acid Extraction
for Manual and Automated Systems

Laboratory Use Only

Instruction Manual
Version 1.1

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TABLE OF CONTENTS

Explanation of Symbols

Catalogue Number

Batch Code

Consult instructions for use

Manufacturer

Use by date

Temperature limit

Contains sufficient for <n> tests

1. INTRODUCTION

The SphaeraMag® Genomic DNA Milk purification kit is designed for the rapid and reliable isolation of genomic DNA (gDNA) from milk samples with a high range of fat content. The gDNA is bound to the surface of paramagnetic beads, while proteins and cellular debris are removed during the washing steps. The gDNA is then eluted in the provided Elution Buffer. The isolated genomic DNA (gDNA) can be used for different downstream applications, such as qPCR, end-point PCR, screening and detection of potential pathogens and other microorganisms.

2. KIT COMPONENTS

	SphaeraMag® Genomic DNA Milk Purification Kit - Universal 96	SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 96	SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 32	SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled Mini
Catalogue No.	**PCCSKU16105**	**PCCSKU16103**	**PCCSKU16102**	**PCCSKU16104**
Preps	96	96	80	48
Lysis/Binding Buffer	70 ml	Pre-filled	Pre-filled	Pre-filled
Wash Buffer I	47 ml	Pre-filled	Pre-filled	Pre-filled
Wash Buffer II	27 ml	Pre-filled	Pre-filled	Pre-filled
Elution Buffer	15 ml	Pre-filled	Pre-filled	Pre-filled
Magnetic Beads	2 x 1 ml	2 x 1 ml	2 x 1 ml	1 ml
Tip comb(s)	-	1 x 96 tip-comb (in holder)	10 x 8-tip combs	30 x 8 tip-combs

Table 1. Components of SphaeraMag® Genomic DNA Milk Purification Kit.

3. 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 Milk Purification Kit compatibility.

4. STORAGE AND STABILITY

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

5. PRECAUTION

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

6. PROCEDURE OVERVIEW

The procedure starts with the Lysis/Binding step, where a specifically formulated chaotropic salt-based Lysis/Binding buffer is used to simultaneously lyse cells, degrade protein components, and bind the gDNA to the surface of the paramagnetic beads.

A three-step washing procedure, including two washing buffers, is performed for the removal of proteins, detergents, and other impurities. During the last step, gDNA is released from the magnetic beads using the Elution buffer.

7. ILLUSTRATED PROTOCOL

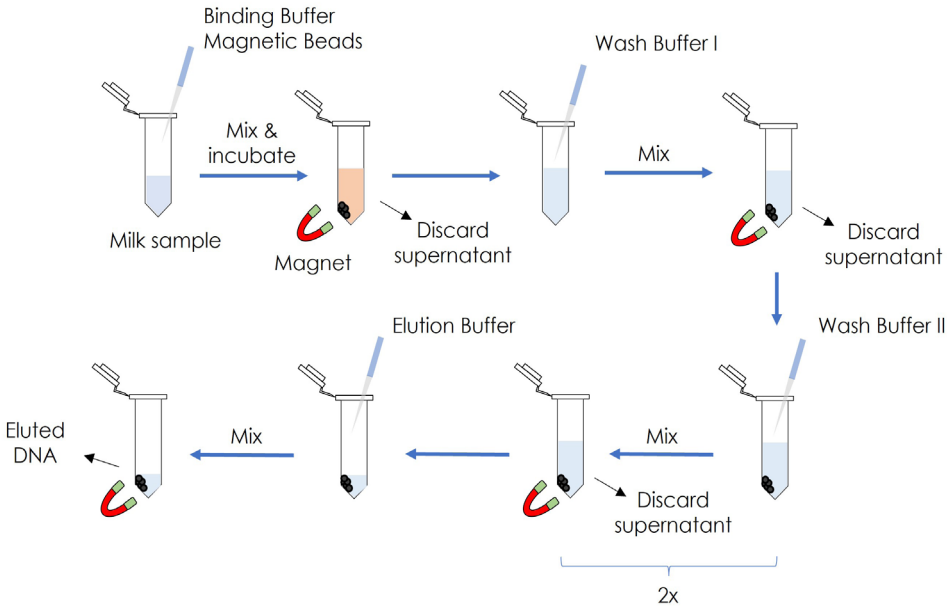

Figure 1. Illustrated protocol for manual gDNA isolation.

8. REQUIRED MATERIALS NOT SUPPLIED

General

- Ethanol, absolute (only for Universal 96)
- Isopropanol, ≥99.5% (only for Universal 96)
- Suitable reagents and sample dispensing options
- Consumables for isolation devices for automated extraction (e.g., Deep-well plates, see Table 17)
- Vortexer
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)

Specific for manual purification

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

9. PERFORMANCE AND DOWNSTREAM APPLICATIONS

The SphaeraMag® Genomic DNA Milk Purifcation Kit generates excellent input material regarding DNA integrity and purity for downstream applications, such as quantitative PCR (qPCR).

Quantitative PCR (qPCR)

Genomic DNA (gDNA) from milk samples with different fat contents was used as template for the detection of *Escherichia coli* (*E. coli*) and *Staphylococcus* sp. **Figure 2** shows results obtained from a qPCR assay, using PhoenixDx® Pan Bacteria Kit (Procomcure Biotech #PCCSKU15329), which allows the detection of genomic sequences of *Escherichia coli* (*E. coli*) and *Staphylococcus* sp. The amplification was performed on Applied Biosystems™ QuantStudio 5 qPCR thermal cycler.

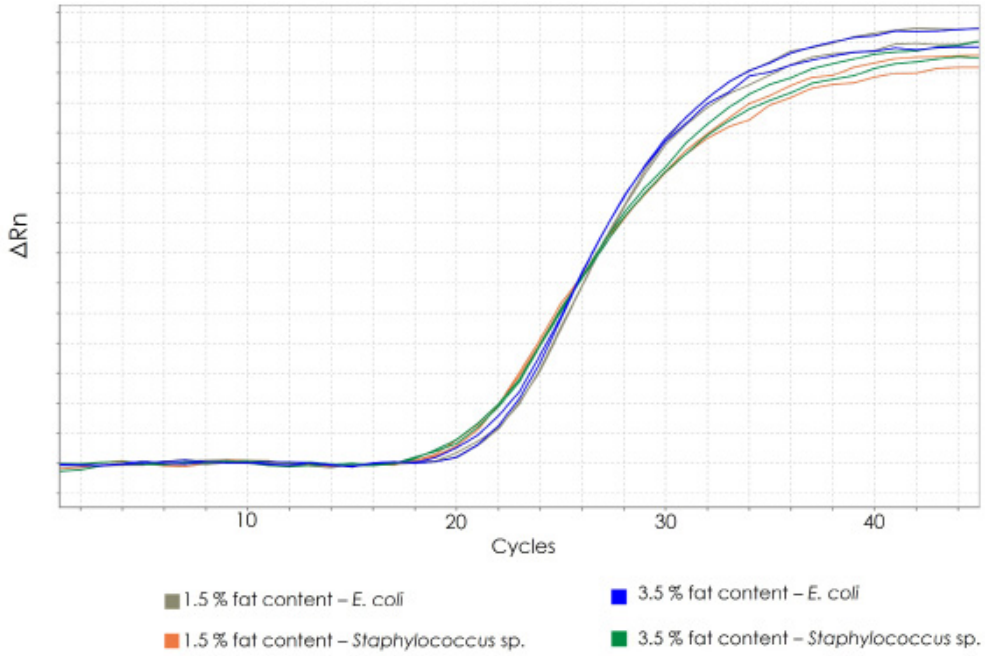

Figure 2. Detection of *Escherichia coli* (*E. coli*) and *Staphylococcus* sp. in gDNA isolated from milk samples with 1.5% and 3.5% fat content.

10. SAMPLE STORAGE AND HANDLING GUIDELINES

Overall quality and prior storage conditions of the starting material can have a high impact on the ability to obtain suitable gDNA. Store milk samples at +4 to +8°C. Exposure to high temperatures (>15°C) may lead to lower yield and/or quality of the isolated gDNA, possibly impairing downstream applications.

11. 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. Store at room temperature.
- Prepare Wash Buffer II (only for Universal 96) by adding Ethanol, absolute (not supplied), as indicated on the bottle label. Store at room temperature.
- Lysis/Binding Buffer and Wash Buffer I may form precipitates when stored under cool conditions (only for Universal 96). Check individually bottled buffers for precipitation before use. Re-dissolve at +37°C and gently mix, if necessary.

12. ELUTION AND STORAGE

Buffer Composition
The Elution Buffer is 10 mM Tris-HCl, pH 8.0. Alternatively, nuclease-free water or other low ionic strength buffers can be used.

Volume
The recommended elution volume is 150 µl. A higher elution volume results in higher yield, but decreased gDNA concentration. A lower volume allows for higher concentration.

Storage
The eluted gDNA can be safely stored at +4 to +8°C for weeks to months. For long term storage, store the eluted gDNA at -80°C. Repeated freeze-thaw cycles should be avoided to maintain the integrity of gDNA.

13. PURIFICATION PROTOCOLS

13.1. AUTOMATED: AUTO-PURE 96/PHOENIX-PURE 96

Compatible Kits: SphaeraMag® Genomic DNA Milk Purification Kit - Universal 96
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 96

Important:

- Vortex the Magnetic Beads thoroughly for 30 sec to fully resuspend them before each use.
- Tip combs and pre-filled plates are for single use only. Do not reuse them.
- Make sure that buffers are at the bottom of each well and not at the sealing foil. If necessary, centrifuge the deep-well plates at 2,000 x *g* for 3 minutes to remove any droplets from the sealing foil.
- The total volume in each well should not exceed 950 µl to avoid spilling and cross-contamination.

1. Make sure to have the ready-to-use V-bottom 96 deep-well plate(s), which contain the following reagents (Table 3):

Step Name	Plate Position	Reagents	Volume per well
Loading	1	Tip comb	-
Lysis/Binding	2	Lysis/Binding Buffer	700 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	150 µl

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

2. Carefully remove the sealing foil from the pre-filled plates.
3. Pipette 200 µl of milk sample and 20 µl Magnetic Beads into the well(s) of Position-2 plate (**orange**; Table 4).

Step Name	Plate Position	Sample/Reagents	Volume per well
Loading	1	Tip comb	-
Lysis/Binding	2	Lysis/Binding Buffer **Sample** **Magnetic Beads**	700 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	150 µl

Table 4. Pipetting scheme for milk samples and Magnetic Bead transfer into the V-bottom 96 deep-well plate for Auto-Pure 96/Phoenix-Pure 96 devices.

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 the program 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 (sec)	2nd segment time (sec)
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Lysis/Bind	2	5	60	0	900	3	40	2	1	3	10	10
3	Wash 1	4	1	60	0	600	5	0	2	1	1	10	10
4	Wash 2	5	1	45	0	600	5	0	2	1	3	5	5
5	Wash 3	6	1	45	2.5	600	5	0	2	1	3	5	5
6	Elution	8	5	90	0	150	3	0	1	3	3	30	1
7	Unload	1	0.2	0	0	900	3	0	1	1	1	1	1

Table 5. Program settings for "SphaeraMag® Genomic DNA Milk Purification" on Phoenix-Pure 96/Auto-Pure 96 devices.

6. Run the program.
7. Once the run is completed, remove the Position-8 Plate (Elution Buffer) from the device and discard the rest of the plates. Transfer eluates from Position-8 plate (Elution Buffer) into new tubes, if necessary. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

13.2. AUTOMATED: KingFisher™ FLEX PURIFICATION SYSTEM

Compatible Kits: SphaeraMag® Genomic DNA Milk Purification Kit - Universal 96
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 96

Important:
- Vortex the Magnetic Beads thoroughly for 30 sec to fully resuspend them before each use.
- Tip combs and pre-filled plates are for single use only. Do not reuse them.
- Make sure that buffers are at the bottom of each well and not at the sealing foil. If necessary, centrifuge the deep-well plates at 2,000 x *g* for 3 minutes to remove any droplets from the sealing foil.
- The total volume in each well should not exceed 950 µl to avoid spilling and cross-contamination.

1. Open the BindIt software of the KingFisher™ Flex Purification system and define the plate names, the plate type (i.e., 96 standard plate) and the reagents (Table 6) in the "Layout" tab.
2. Make sure to have the ready-to-use V-bottom 96 deep-well plate(s), which contain the reagents shown in Table 6.

Suggested step name	Plate labels	Reagents / Samples	Volume per well
Pick up	Position-1 96-Deepwell Plate and Tip Comb	Tip comb	-
Lysis / Binding	Position-2 Lysis/Binding Buffer	Lysis/Binding Buffer **Sample** **Magnetic Beads**	700 µl **200 µl** **20 µl**
Wash 1	Position-4 Wash Buffer I	Wash Buffer I	600 µl
Wash 2	Position-5 & Position-6 Wash Buffer II	Wash Buffer II	600 µl
Wash 3	Position-5 & Position-6 Wash Buffer II	Wash Buffer II	600 µl
Elution	Position-8 Elution Buffer	Elution Buffer	150 µl

Table 6. Setup and pipetting scheme for the transfer of milk samples and Magnetic Beads into 96 deep-well plates compatible with KingFisher™ Flex Purification System.

3. Carefully remove the sealing foil from the pre-filled plates.
4. Pipette 200 µl of milk sample and 20 µl Magnetic Beads (**orange**; Table 6) into the wells of Position-2 (Lysis/Binding Buffer) plate.
5. Load the V-bottom 96 deep-well plates onto the instrument's plate positions according to plate layout (Table 6). **Note:** Make sure that all plates are placed in the correct orientation.
6. Set the program according to Table 7.

Step name	Reagents / Settings	Setting selection
Tip	96 DW tip comb	
1. Pick up	Tip Comb	
2. Lysis/Binding	Lysis/Binding Buffer	
2.1. Beginning of step	Precollect Release beads	No No
2.2. Mixing/heating	Mixing time, speed Heating temperature [°C] Preheat	00:05:00, Medium 40 Yes
2.3. End of step	Postmix Collect count Collect time [s]	No 5 30
3. Collect Beads	Lysis/Binding Buffer	
	Collect count Collect time [s]	5 30
4. Wash 1	Wash Buffer I	
4.1. Beginning of step	Precollect Release beads, speed	No 00:00:20, Medium
4.2. Mixing/heating	Mixing time, speed Heating during mixing	00:01:00, Medium No
4.3. End of step	Postmix Collect count Collect time [s]	No 4 30

Step name (cont.)	Plate names / Settings	Setting selection
5. Wash 2	Wash Buffer II	
5.1. Beginning of step	Precollect Release beads	No Yes
5.2. Mixing/heating	Mixing time, speed Heating during mixing	00:01:00, Medium No
5.3. End of step	Postmix Collect count Collect time [s]	No 4 30
6. Wash 3	Wash Buffer II	
6.1. Beginning of step	Precollect Release beads	No Yes
6.2. Mixing/heating	Mixing time, speed Heating during mixing	00:01:00, Medium No
6.3. End of step	Postmix Collect count Collect time [s]	No 4 30
7. Dry	Wash Buffer II	
	Dry time Tip position	00:03:00 Outside well / tube
8. Elution	Elution Buffer	
8.1. Beginning of step	Precollect Release beads, speed	No 00:00:20, Medium
8.2. Mixing/heating	Shake 1 time, speed Shake 2 time, speed Heating during mixing	00:05:00, Bottom mix 00:00:45, Medium No
8.3. End of step	Postmix Collect count Collect time [s]	No 1 30
9. Collect Beads*	Elution Buffer	
9.1. Beginning of step	Precollect Release beads	No No
9.2. Mixing/heating	Mixing Heating during mixing	[none] No
9.2. End of step	Postmix Collect count Collect time [s]	No 3 1
10. Leave	Tip Comb Plate	

*This step is optional and can be modified for the collection of beads at the final elution step. Alternatively the time of bead collection at Step 8 (Elution) can be increased.

Table 7. Program settings for "SphaeraMag® Genomic DNA Milk Purification" on KingFisher™ Flex Purification device (96 deep-well plates).

7. Run the program.
8. Once the run is completed, remove the Elution Plate from the device and discard the rest of the plates.
9. Process the eluates directly, or store at +4 to +8°C for short-term or at -80°C for long-term storage.

13.3. AUTOMATED: AUTO-PURE 32/PHOENIX-PURE 32

Compatible Kit: SphaeraMag® Genomic DNA Milk Purification Kit - Universal 96
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 32

Important:

- Vortex the Magnetic Beads thoroughly for 30 sec to fully resuspend them before each use.
- Tip combs and pre-filled plates are for single use only. Do not reuse them.
- Make sure that buffers are at the bottom of each well and not at the sealing foil. If necessary, centrifuge the deep-well plates at 2,000 x *g* for 3 minutes to remove any droplets from the sealing foil.
- The total volume in each well should not exceed 950 µl to avoid spilling and cross-contamination.

1. Make sure to have the ready-to-use U-bottom 96 deep-well plate(s), which contain the following reagents (Table 8).

Well	Reagents	Volume per well
Column 1/7	Lysis/Binding Buffer	700 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	150 µl

Table 8. Setup for a U-bottom 96 deep-well plate compatible with Auto-Pure 32/Phoenix-Pure 32 devices.

2. Carefully remove the sealing foil from the U-bottom 96 deep-well plate(s).
3. Pipette 200 µl of milk sample and 20 µl Magnetic Beads into the well(s) of Columns 1 and 7 (**orange**; Table 9).
4. Load the prepared U-bottom 96 deep-well plate onto the instrument. **Note:** Make sure that the plates are placed in the correct orientation.

Well	Reagents	Volume per well
Column 1/7	Lysis/Binding Buffer **Sample** **Magnetic Beads**	700 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	150 µl

Table 9. Pipetting scheme for the transfer of milk samples and Magnetic Beads into a U-bottom 96 deep-well plate compatible with Auto-Pure 32/Phoenix-Pure 32 devices.

5. Place new clean tip combs into the instrument.
6. Set the program in the Auto-Pure 32/Phoenix-Pure 32 device(s) 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 amp (%)	magnet pos (%)	magnet speed (1-10)
1	Lysis / Bind	1	5	60	0	900	3	40	0	80	0	3
2	Wash 1	3	1	60	0	600	5	0	0	80	0	3
3	Wash 2	4	1	45	0	600	5	0	0	80	0	3
4	Wash 3	5	1	45	2.5	600	5	0	0	80	0	3
5	Elution	6	5	90	0	150	3	0	0	80	0	3
6	Waste	1	0.2	0	0	900	3	0	0	80	0	3

Table 10. Program settings for "SphaeraMag® Genomic DNA Milk Purification" with Auto-Pure 32/Phoenix-Pure 32 devices.

7. Run the program.
8. After the run is completed, remove the U-bottom 96 deep-well plate(s), and discard the tip combs. Transfer the eluates from the columns 6 and 12 into new tubes or plates (not provided), if necessary. Process the eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

13.4. AUTOMATED: AUTO-PURE MINI

Compatible Kit: SphaeraMag® Genomic DNA Milk Purification Kit - Universal 96
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled Mini

IImportant:
- Vortex the Magnetic Beads thoroughly for 30 sec to fully resuspend them before each use.
- Tip combs and pre-filled plates are for single use only. Do not reuse them.
- Make sure that buffers are at the bottom of each well and not at the sealing foil. If necessary, centrifuge the deep-well plates at 2,000 x *g* for 3 minutes to remove any droplets from the sealing foil.
- The total volume in each well should not exceed 950 µl to avoid spilling and cross-contamination.

Samples can be processed in **(a)** cartridges (1 cartridge per sample) or in **(b)** a V-bottom 96 deep-well plate (16 samples per plate). Make sure to have the suitable reagents according to the sample format used, as shown in Tables 11 and 12.

(a) Cartridge format:

Well	Reagents	Volume per well
Position 1	Lysis/Binding Buffer	700 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	150 µl

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

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

Well	Reagents	Volume per well
Column 1/7	Lysis/Binding Buffer	700 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	150 µl

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

1. Carefully remove the sealing foil from the cartridge(s) or from the V-bottom 96 deep-well plate.
2. Transfer 200 µl of milk samples and 20 µl of Magnetic Beads (**orange**) into the appropriate wells according to the method used, as shown in Tables 13 and 14.

(a) Cartridge format:

Well	Samples/Reagents	Volume per well
Position 1	Lysis/Binding Buffer **Sample** **Magnetic Beads**	700 µl **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	150 µl

Table 13. Pipetting scheme for the transfer of milk samples and Magnetic Beads into cartridge(s).

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

Well	Samples/Reagents	Volume per well
Column 1/7	Lysis/Binding Buffer **Sample** **Magnetic beads**	700 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	150 µl

Table 14. Pipetting scheme for the transfer of milk samples and Magnetic Beads into the V-bottom 96 deep-well plate.

3. Load the cartridge(s) or the V-bottom 96 deep-well plate onto the instrument. **Note:** Make sure that the cartridge(s) or the V-bottom 96 deep-well plate are placed in the correct orientation.
4. Place new clean tip comb(s) into the instrument.
5. Set the program according to Table 15.
6. Run the program.

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	Lysis / Bind	1	5	60	0	900	3	40	0	80	0	3
2	Wash 1	3	1	60	0	600	5	0	0	80	0	3
3	Wash 2	4	1	45	0	600	5	0	0	80	0	3
4	Wash 3	5	1	45	2.5	600	5	0	0	80	0	3
5	Elution	6	5	90	0	150	3	0	0	80	0	3
6	Waste	1	0.2	0	0	900	3	0	0	80	0	3

Table 15. Program settings for "SphaeraMag® Genomic DNA Milk Purification" on Auto-Pure Mini device.

7. Once the run is completed, remove the cartridge(s) or the V-bottom 96 deep-well plate and discard the tip comb(s). Transfer the eluate(s) from column(s) 6/12, or from position 6, into new tubes (not provided), if necessary. Process the eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

13.5. MANUAL PURIFICATION

Preparation:

- Pre-heat the appropriate volume of Lysis/Binding Buffer at 40°C.
- Vortex the Magnetic Beads thoroughly before each use. Make sure that the magnetic beads are completely resuspended.

1. Transfer 200 µl of milk sample into a nuclease-free 1.5 ml or 2 ml microcentrifuge tube (not provided).
2. Add 700 µl Lysis/Binding Buffer. Mix by pipetting up and down at least 5 times.
3. Add 20 µl Magnetic Beads. Mix by pipetting up and down at least 10 to 15 times.
4. Incubate for 5 minutes at room temperature. **Note:** Make sure that the beads stay fully mixed in the sample by constantly inverting the tube(s).
5. Place the tube on a magnetic stand. Allow the magnetic beads to settle completely.
6. Remove and discard the supernatant. Do not disturb the magnetic bead pellet.
7. Add 600 µl Wash Buffer I. **Note:** Make sure that the correct amount of Isopropanol (≥99.5 %) is added to Wash Buffer I.
8. Remove the tube from the magnetic stand. Mix well by pipetting up and down several times.
9. Place the tube on the magnetic stand. Allow the beads to settle in the magnet until the solution is completely clear.
10. Remove and discard the supernatant. Do not disturb the magnetic bead pellet.
11. Add 600 µl Wash Buffer II. **Note:** Make sure that the correct amount of Ethanol (absolute) is added to Wash Buffer II.
12. Remove the tube from the magnetic stand. Mix well by pipetting up and down several times.
13. Remove and discard the supernatant. Do not disturb the magnetic bead pellet.
14. Repeat steps 11-13 for an additional washing step with Wash Buffer II.
15. Remove any residual liquid. Dry the magnetic beads by leaving the lids of the tubes open for 5 minutes at room temperature.
16. Add 150 µl Elution Buffer to the tube.
17. Remove the tube from the magnetic stand. Mix very well by pipetting up and down at least 10 times, until the beads are completely resuspended. **Note:** Incomplete mixing can lead to low yields.
18. Incubate at room temperature for 1 minute.
19. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
20. Transfer the eluate into a clean microcentrifuge tube (not provided). Process directly, store at +4 to +8°C for short-term or at -80°C for long-term storage.

15. TROUBLESHOOTING GUIDE

Please use this guide to troubleshoot 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 the Magnetic Beads.	Thoroughly resuspend by vortexing the Magnetic Beads before use.
	DNA degradation during sample handling or storage.	Process the sample immediately after collection or after removing them from the recommended storage conditions.
	Incorrect preparation of Wash Buffers.	Prepare Wash Buffers by adding the correct amounts of Ethanol (Wash Buffer II) or Isopropanol (Wash Buffer I) according to instructions indicated on the bottle labels.
Problems with downstream applications	Poor DNA quality.	Do not freeze-thaw the isolated gDNA more than once and do not store at room temperature.
	Insufficient DNA was used.	Quantify the isolated gDNA accurately. Use a sufficient amount of gDNA, for downstream applications.
	Ethanol carry-over.	Dry the Magnetic Beads completely before the Elution Step.
Carry-over of magnetic beads	Incomplete magnetization.	Place the eluted samples on a magnetic stand device for additional 5 minutes or centrifuge at >4,000 x *g* for 5 minutes. When using automated isolation, check the instrument settings and increase bead binding time, if necessary.

Table 16. Troubleshooting guide.

16. CONSUMABLES AND RELATED PRODUCTS

Product	Content	Cat.No.
PhoenixDx® Pan Bacteria	96 rxn	PCCSKU15329
PhoenixDx® Pan Fungi	96 rxn	PCCSKU15330
SphaeraMag® Genomic DNA Milk Purification Kit - Universal 96	96 preps	PCCSKU16105
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 96	96 preps for Phoenix-Pure 96 & Auto-Pure 96	PCCSKU16103
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled 32	80 preps for Phoenix-Pure 32 & Auto-Pure 32	PCCSKU16102
SphaeraMag® Genomic DNA Milk Purification Kit - Pre-filled Mini	48 preps for Auto-Pure Mini	PCCSKU16104
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

Table 17. Consumables and related products.

Ordering information

For ordering SphaeraMag® Genomic DNA Milk Purification Kit and other products, visit us at

www.procomcure.com

or order via E-mail:

sales@procomcure.com

PRO COM CURE

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Quality Management System Certified
ISO 9001:2015
EN ISO 13485:2016