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# SphaeraMag<sup>®</sup> Genomic DNA Saliva Purification Kit

Nucleic Acid Extraction  
for Manual and Automated Systems

Laboratory Use Only

Instruction Manual  
Version 1.6

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## 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 Saliva Purification Kit is designed for rapid and reliable isolation of genomic DNA (gDNA) from saliva and oral swab samples. Samples are lysed in specifically formulated buffers to release nucleic acids. The gDNA is bound to the surface of paramagnetic beads, while proteins and cellular debris are removed during washing steps. The gDNA 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 gDNA is suitable for numerous downstream applications such as qPCR, end-point PCR, SNP analysis, genotyping, microarray analysis and library preparation for NGS sequencing.

## 2. KIT COMPONENTS

	SphaeraMag® Genomic DNA Saliva Purification Kit - Universal 96	SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled 96	SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled 32	SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled Mini
Catalogue No.	<b>PCCSKU16081</b>	<b>PCCSKU16082</b>	<b>PCCSKU16083</b>	<b>PCCSKU16084</b>
Preps	96	96	80	48
Enhancer Solution	4 ml	pre-filled	pre-filled	pre-filled
Lysis/Binding Buffer	40 ml	40 ml	40 ml	20 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 1 ml	2 x 1 ml	1 ml
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.** SphaeraMag® Genomic DNA Saliva Purification Kit content.

## 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 Saliva Purification Kit compatibility.

## 4. STORAGE AND STABILITY

Proteinase K enzyme must be stored at -25 to -15°C, Magnetic Beads 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 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.

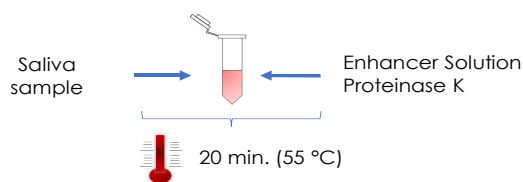
## 6. PROCEDURE OVERVIEW

Saliva and oral swab samples in solution are first 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 gDNA to the surface of paramagnetic beads. Additionally, it 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 gDNA is then released from the magnetic beads using Elution Buffer.

## 7. ILLUSTRATED PROTOCOL

### PART 1: SAMPLE DIGEST



### PART 2: LYSIS/BINDING AND ELUTION

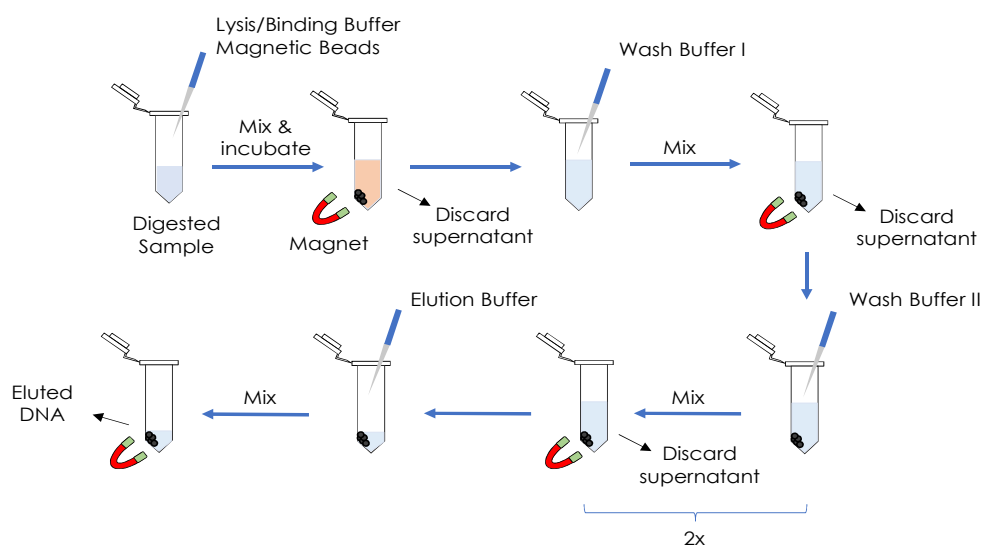


Figure 1. Illustrated protocol for manual gDNA isolation from saliva samples.

## 8. REQUIRED MATERIALS NOT SUPPLIED

### General

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

### 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



## 9. SPECIFICATIONS

<b>Sample input</b>	Up to 400 µl saliva/oral swab sample in solution
<b>Yield</b>	Saliva sample: ~6-12 µg; oral swab sample: ~3-5 µg
<b>Genomic DNA Size</b>	Peak size >23 kb
<b>Purity</b>	A260/280 >1.8 A260/230 >2.0

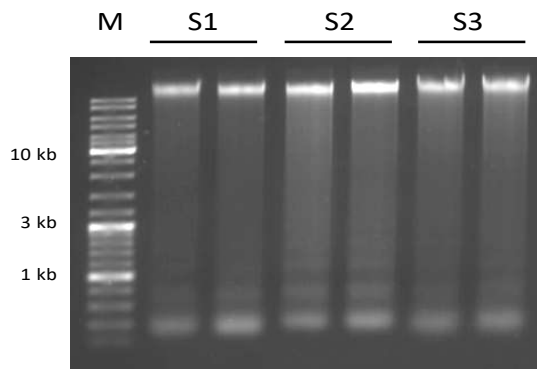
**Table 3.** Kit specifications.

## 10. PERFORMANCE AND DOWNSTREAM APPLICATIONS

The SphaeraMag® Genomic DNA Saliva Purification Kit generates excellent input material regarding purity for downstream applications such as quantitative PCR. In general, saliva naturally leads to lower integrity due to partial presence of dead cell debris, and thus degraded gDNA.

### DNA Integrity

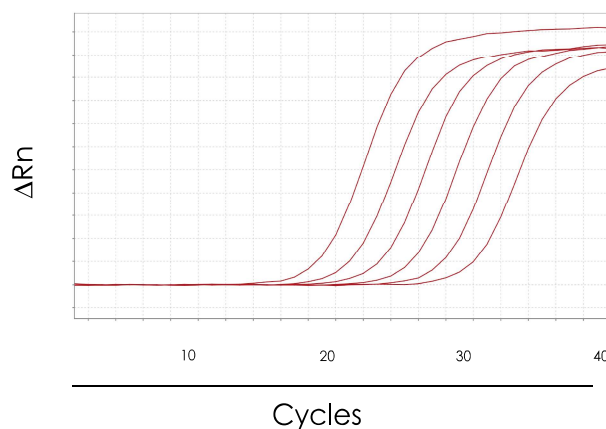
gDNA extracted from representative **(S1)** oral swab samples in 1x PBS , **(S2)** oral swab samples in PhoenixProtect DNA/RNA Conservation Solution (Procomcure Biotech #PCCSKU16009) and **(S3)** saliva samples were analyzed regarding DNA integrity by loading 10 µl eluate on an 1% agarose gel (figure 2).



**Figure 2.** Integrity of gDNA isolated from swab and saliva samples assessed by agarose gel electrophoresis.

### Quantitative PCR

Purified gDNA from representative human saliva samples was diluted to produce a four-log range of input template concentrations. Results were obtained using primers targeting human-specific genomic sequence for qPCR assays with the PhoenixDx® ABC qPCR Mastermixes (Procomcure Biotech #PCCSKU12011) and cycled on Applied Biosystems™ QuantStudio 5 qPCR thermal cycler. Results confirm that the eluted gDNA is highly pure and free from inhibitors, optimal for qPCR.



**Figure 3.** qPCR data using human-specific designed assays.

## 11. SAMPLE STORAGE AND HANDLING GUIDELINES

The SphaeraMag Genomic DNA Saliva Purification Kit isolates gDNA from preserved (e.g. using a stabilizing reagent such as PhoenixProtect DNA/RNA Conservation Solution, Procomcure Biotech #PCCSKU16009 or a preservative reagent), fresh saliva samples and oral swab samples. To ensure a high quality gDNA, fast processing of samples is in general highly recommended. When isolating from preserved or stabilized saliva or oral swab samples, get familiar with manufacturer recommendations regarding sample handling. Many saliva collection systems may require pre-treatment before the actual sample processing. When working with dry oral swab samples, reconstitution in a suitable solution (e.g. 1x PBS or PhoenixProtect DNA/RNA Conservation Solution) is highly recommended.

## 12. 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  $\geq 99.5\%$  (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  $+37^{\circ}\text{C}$  and gently mix, if necessary.

## 13. 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.

### Temperature

Elution is strongly recommended with the Elution Buffer pre-heated to  $+60^{\circ}\text{C}$  to improve elution efficiency.

### Volume

The recommended elution volume ranges between 50-100  $\mu\text{l}$ . 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  $+8^{\circ}\text{C}$  for weeks to months. For long term storage, store the eluted gDNA at  $-80^{\circ}\text{C}$ . Repeated freeze-thaw cycles should be avoided to maintain eluate integrity.

## 14. EXTRACTION PROTOCOLS

### 14.1. AUTOMATED: AUTO-PURE 96/PHOENIX-PURE 96

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

**Important:**

- 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 plates at 2,000 xg 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 4) 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	100 µl

**Table 4.** 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 5) 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 <b>Sample</b> <b>Proteinase K</b>	40 µl <b>up to 400 µl</b> <b>20 µl</b>
-	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	100 µl

**Table 5.** 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 5). Note: Make sure that all plates are placed in the correct orientation.
5. Set program 1 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)	1 <sup>st</sup> segment time	2 <sup>nd</sup> segment time
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Digest	2	20	80	0	500	3	55	2	1	3	15	15
3	Unload	1	0	1	0	5	1	0	1	1	1	1	1

**Table 6.** Program setting for step 1 of 2 for “Genomic DNA Saliva Purification” on 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 400 µl Lysis/Binding Buffer + 20 µl Magnetic Beads.  
Note: Ensure that beads stay fully mixed by constantly inverting the tube.
9. Transfer 420 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 7.

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)	1 <sup>st</sup> segment time (sec)	2 <sup>nd</sup> segment time (sec)
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Lysis/Bind	2	5	80	0	900	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 7.** Program setting for step 2 of 2 for “Genomic DNA Saliva Purification” on 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.

## 14.2. AUTOMATED: AUTO-PURE 32/PHOENIX-PURE 32

**Compatible Kits:** SphaeraMag® Genomic DNA Saliva Purification Kit - Universal 96  
SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled 32

**Important:**

- 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 2,000 xg 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 8) 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	100 µl

**Table 8.** 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 plates.
3. Transfer sample(s) and Proteinase K (**orange**) to appropriate well(s) in columns 1/7 (Table 9).  
Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

Well	Samples/Reagents	Volume per well
Column 1/7	Enhancer Solution <b>Sample</b> <b>Proteinase K</b>	40 µl <b>up to 400 µl</b> <b>20 µl</b>
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	100 µl

**Table 9.** Pipetting scheme for sample and Proteinase K transfer into U-bottom 96 deep-well plates prior to program 1 of 2.

4. Load U-bottom 96 deep-well plate(s) onto the instrument. Note: Make sure that each plate(s) 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 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	Digest	1	20	68	0	500	7	55	0	80	0	3

**Table 10.** Program setting for step 1 of 2 for "Genomic DNA Saliva Purification" on 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 amount of reactions. For each sample: Mix 400 µl Lysis/Binding Buffer + 20 µl Magnetic Beads.  
Note: Ensure that beads stay fully mixed by constantly inverting the tube.
10. Transfer 420 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 11.

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	68	0	900	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	1	0	0	80	0	3

**Table 11.** Program setting for step 2 of 2 for "Genomic DNA Saliva Purification" on 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.

### 14.3. AUTOMATED PURIFICATION: AUTO-PURE MINI

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

**Important:**

- 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 2,000 xg 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 12 or Table 13.

**(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	100 µl

**Table 12.** 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	100 µl

**Table 13.** 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 14 or Table 15.  
Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

**(a) Cartridge format:**

Well	Samples/Reagents	Volume per well
Position 1	Enhancer Solution <b>Sample</b> <b>Proteinase K</b>	40 µl <b>up to 400 µl</b> <b>20 µl</b>
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	100 µl

**Table 14.** 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	Samples/Reagents	Volume per well
Column 1/7	Enhancer Solution <b>Sample</b> <b>Proteinase K</b>	40 µl <b>up to 400 µl</b> <b>20 µl</b>
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	100 µl

**Table 15.** 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 16.

step	step name	hole site	volume (µl)	mix time (min)	mix speed	dry time (min)	temperature (°C)	segments	every time (sec)	magnetization time (sec)	cycle	magnet speed (mm/sec)	mix scope (%)	mix pos.	magnet pos.
1	Digest	1	500	20	8	0	55	2	30	0	1	3	80	0	0
2	Waste	1	500	0.1	5	0	0	1	0	0	1	2.5	80	0	0

**Table 16.** Program setting for step 1 of 2 for "Genomic DNA Saliva Purification" on Auto-Pure Mini device.

6. Run the program.
7. Once the run is finished, leave tip comb(s) in 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 amount of reactions. For each sample: Mix 400  $\mu$ l Lysis/Binding Buffer + 20  $\mu$ l Magnetic Beads.  
Note: Ensure that beads stay fully mixed by constantly inverting the tube.
9. Transfer 420  $\mu$ l 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 17.

step	step name	well	mix time (min)	magnet (sec)	wait time (min)	volume ( $\mu$ l)	mix speed (1-10)	temp ( $^{\circ}$ C)	mix pos (%)	mix amp (%)	magnet pos (%)	magnet speed (1-10)
1	Lysis/Bind	1	5	68	0	900	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	1	0	0	80	0	3

**Table 17.** Program setting for step 2 of 2 for "Genomic DNA Saliva Purification" on 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 $^{\circ}$ C for short-term or at -80 $^{\circ}$ C for long-term storage.



## 14.4. MANUAL EXTRACTION

### Preparation:

- Pre-heat appropriate volume of Elution Buffer to +60°C.
1. Transfer 40 µl Enhancer Solution to a nuclease-free 1.5 ml or 2 ml microcentrifuge tube.
  2. Add up to 400 µl saliva or reconstituted oral swab sample and 20 µl Proteinase K to the tube. Mix by pipetting up and down at least 5 times.
  3. 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.
  4. Vortex Magnetic Beads thoroughly. Combine them with the Lysis/Binding Buffer in a nuclease-free tube for required amount of reactions. For each sample: Mix 400 µl Lysis/Binding Buffer + 20 µl Magnetic Beads. Note: Ensure that beads stay fully mixed by constantly inverting the tube.
  5. Pipette 420 µl Lysis/Binding Buffer and Magnetic Bead mix to the processed sample.
  6. 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.
  7. Place the tube on a magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
  8. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
  9. Add 600 µl Wash Buffer I.
  10. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
  11. Place the tube on the magnetic stand. Allow Magnetic beads to settle until the solution is completely clear.
  12. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
  13. Add 600 µl Wash Buffer II.
  14. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
  15. Place the tube on the magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
  16. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
  17. Repeat steps 13-16 for an additional washing step with Wash Buffer II.
  18. Remove any residual liquid. Leave the tube open for 5 min to allow Magnetic Beads air dry.
  19. Add 100 µl pre-heated Elution Buffer to the tube.
  20. Remove the tube from the magnetic stand. Mix well by pipetting up and down ~10 times.
  21. Incubate at room temperature for 1 min.
  22. Place the tube on the magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
  23. Transfer the eluate to a clean tube. 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 [support@procomcure.com](mailto:support@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.
	Frozen samples not mixed properly after thawing	Thaw frozen samples on ice and mix the sample by gently inverting the tube.
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 >4,000 xg for 5 minutes. When using automated isolation, check the instrument settings and increase bead binding time, if necessary.

**Table 18.** Troubleshooting guide.

## 16. CONSUMABLES AND RELATED PRODUCTS

Product	Content	Cat.No.
SphaeraMag® Genomic DNA Saliva Purification Kit - Universal 96	96 preps	PCCSKU16081
SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled 96	96 preps for Phoenix-Pure 96 & Auto-Pure 96	PCCSKU16082
SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled 32	80 preps for Phoenix-Pure 32 & Auto-Pure 32	PCCSKU16083
SphaeraMag® Genomic DNA Saliva Purification Kit - Pre-filled Mini	48 preps for Auto-Pure Mini	PCCSKU16084
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 19.** Consumables and related products.

### *Ordering information*

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

[www.procomcure.com](http://www.procomcure.com)

or order via E-mail:

**[sales@procomcure.com](mailto:sales@procomcure.com)**



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