# SphaeraMag® Genomic DNA Hair 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 Hair Purification Kit is designed for rapid and reliable isolation of genomic DNA (gDNA) from hair samples. Samples are lysed in a specifically formulated buffer 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 Hair Purification Kit - Universal 96 | SphaeraMag® Genomic DNA Hair Purification Kit - Pre-filled 96 | SphaeraMag® Genomic DNA Hair Purification Kit - Pre-filled 32 | SphaeraMag® Genomic DNA Hair Purification Kit - Pre-filled Mini |
|---|---|---|---|---|
| Catalogue No. | **PCCSKU16094** | **PCCSKU16095** | **PCCSKU16096** | **PCCSKU16097** |
| Preps | 96 | 96 | 80 | 48 |
| Lysis Buffer | 20 ml | 20 ml | 20 ml | 10 ml |
| Binding Buffer | 20 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 | 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 | 1 ml | 1 ml | 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 Hair 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 Hair Purification Kit compatibilty.

# 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.

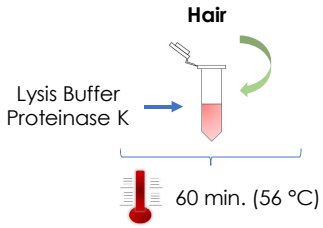
# 6. PROCEDURE OVERVIEW

Hair samples are lysed in a chaotropic salt-based Lysis Buffer to release the gDNA. In the following step, a Binding Buffer is added to allow binding of the gDNA to the surface of paramagnetic beads. A three step washing procedure, including two washing buffers, is performed to remove proteins, detergents and other impurities. The gDNA is then released from the Magnetic Beads using Elution Buffer.

To prevent the co-isolation of RNA during the gDNA extraction, an optional RNase A (not supplied) treatment can be included in the protocol.

# 7. ILLUSTRATED PROTOCOL

**PART 1: SAMPLE LYSIS**




**PART 2: BINDING AND ELUTION**

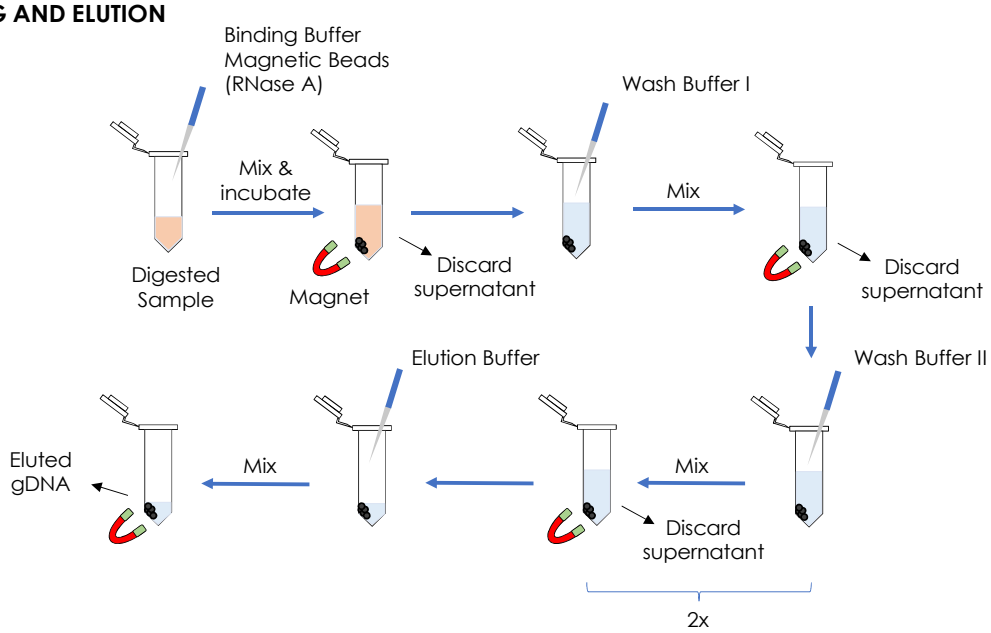



**Figure 1.** Illustrated protocol for manual gDNA isolation.

# 8. REQUIRED MATERIALS NOT SUPPLIED

**General**

- Ethanol, absolute (only for Universal 96)
- Isopropanol ≥99.5% (only for Universal 96)
- (Optional) RNase A - recommendation: 20 mg/ml solution
- Suitable reagent and sample dispensing options
- Consumables for isolation devices for automated extraction
- Vortexer
- Thermal mixer for 1.5 ml or 2 ml tubes (or alternative heating device)
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)

**Specific for manual purification**

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

# 9. SPECIFICATIONS

| | |
|---|---|
| **Recommended sample input** | Hair (incl. bulbs): 20 pcs |
| **Yield** | 8-14 µg |
| **Genomic DNA size** | Peak size >50 kb |
| **Purity** | A260/280 >1.8<br>A260/230 >2.0 |

**Table 3.** Kit specifications.

# 10. PERFORMANCE AND DOWNSTREAM APPLICATIONS

The SphaeraMag® Genomic DNA Hair Purification Kit generates excellent input material regarding DNA integrity and purity for downstream applications such as quantitative PCR.

**DNA Integrity**

The gDNA samples were analyzed regarding DNA integrity by loading 10 µl eluate on an 1% agarose gel. Representative gDNA samples obtained from human hair are shown.

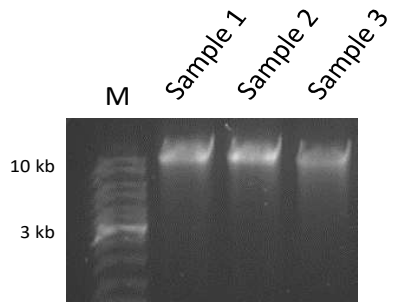



**Figure 2.** Integrity of gDNA isolated from human hair assessed by agarose gel electrophoresis.

**Quantitative PCR**

Purified gDNA from a representative human hair sample 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.

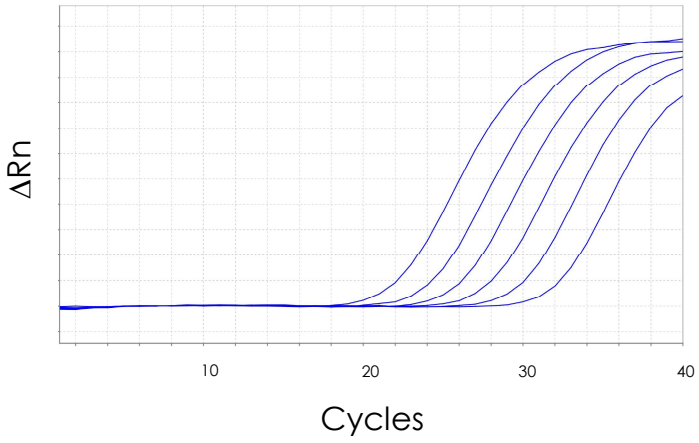



**Figure 3.** qPCR data using human-specific designed assays.

# 11. SAMPLE STORAGE AND HANDLING GUIDELINES

### Sample Storage

To ensure a high quality of isolated gDNA, fast processing of samples is highly recommended. Overall quality and prior storage conditions of the starting material have a high impact on the ability to obtain suitable gDNA. Exposure to e.g. high temperatures for a considerable period of time may lead to diminished yield and/or (partly) unusable isolated gDNA regarding downstream applications.

- Storage and preservation of hair roots and bulbs is highly recommended under dry conditions
- Short-term storage: refrigerated at +4 to +8°C or frozen at -20°C or lower (avoid defrosting)
- Long-term storage: -80°C

### Sample Handling

To ensure optimal yield of isolated gDNA, use the recommended amount of starting material (Table 3). In order to obtain optimal results, cut hairs including bulbs into smallest possible pieces and transfer to a reaction tube.

# 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 ≥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) during initial lysis step.
- Lysis Buffer, Binding Buffer and Wash Buffer I 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.
- For the optional treatment with RNase A (not supplied), prepare an appropriate working solution - recommended concentration: 20 mg/ml RNase A.

# 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°C to improve elution efficiency.

**Volume**

The recommended elution volume ranges between 50-100 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 eluate integrity.

# 14. EXTRACTION PROTOCOLS

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

**Compatible Kits:** SphaeraMag® Genomic DNA Hair Purification Kit - Universal 96
SphaeraMag® Genomic DNA Hair 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 min to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.
- Set a thermal mixer or alternative heating device to +56°C.

1. Make sure to have V-bottom 96 deep-well plates containing buffers (Table 4) ready for use.

| Step Name | Plate Position | Reagents | Volume per well |
|---|---|---|---|
| Loading | 1 | Tip comb | - |
| Binding | 2 | Binding Buffer | 200 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. For each processed sample, transfer required amount of starting material to a 1.5 ml or 2 ml microcentrifuge tube: Up to 20 hairs incl. bulbs (cut into smallest possible pieces).

3. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
4. Incubate in a thermal mixer at +56°C for 60 min with agitation at full speed (~1,400 *rpm*).
5. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 *rpm*) for 5 min.
6. Remove sealing foil from pre-filled plates.
7. Transfer lysate(s) and Magnetic Beads (**orange**) to appropriate well(s) (Table 5) on Position-2 Plate (Binding Buffer).

| Step Name | Plate Position | Sample/Reagents | Volume per well |
|---|---|---|---|
| Loading | 1 | Tip comb | - |
| Binding | 2 | Binding Buffer<br>**Lysate**<br>**Magnetic Beads** | 200 µl<br>**~220 µl**<br>**10 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 5.** Pipetting scheme for lysate and Magnetic Bead transfer into V-bottom 96 deep-well plates.

8. Load V-bottom 96 deep-well plates onto instrument's plate positions according to plate layout (Table 6). Note: Make sure that all plates are placed in the correct orientation.
9. Set program 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 (sec) | 2nd segment time (sec) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Load | 1 | 0 | 1 | 0 | 5 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| 2 | 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 "Genomic DNA Hair Purification" on Phoenix-Pure 96/Auto-Pure 96 device.

10. Run the program.
11. After the run is finished, remove V-bottom 96 deep-well plates and 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: PHOENIX-PURE 32/AUTO-PURE 32

**Compatible Kits:** SphaeraMag® Genomic DNA Hair Purification Kit - Universal 96
SphaeraMag® Genomic DNA Hair 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 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.
- Set a thermal mixer or alternative heating device to +56°C.

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

| Well | Reagents | Volume per well |
|---|---|---|
| Column 1/7 | Binding Buffer | 200 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 7.** Setup for an U-bottom 96 deep-well plate compatible with Phoenix-Pure 32/Auto-Pure 32 device.

2. For each processed sample, transfer required amount to a 1.5 ml or 2 ml microcentrifuge tube: Up to 20 hairs incl. bulbs (cut into smallest possible pieces).

3. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
4. Incubate in a thermal mixer at +56°C for 60 min with agitation at full speed (~1,400 *rpm*).
5. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 *rpm*) for 5 min.
6. Remove sealing foil from pre-filled plates.
7. Transfer lysate(s) and Magnetic Beads (**orange**) to appropriate well(s) in columns 1/7 (Table 8).

| Well | Sample/Reagents | Volume per well |
|---|---|---|
| Column 1/7 | Binding Buffer<br>**Lysate**<br>**Magnetic Beads** | 200 µl<br>**~220 µl**<br>**10 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.** Pipetting scheme for lysate and Magnetic Bead transfer into an U-bottom 96 deep-well plate.

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

9. Set program 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 | 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 | 1 | 0 | 0 | 80 | 0 | 3 |

**Table 9.** Program setting for "Genomic DNA Hair Purification" on Phoenix-Pure 32/Auto-Pure 32 device.

10. Run the program.

11. 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 Hair Purification Kit - Universal 96
SphaeraMag® Genomic DNA Hair 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 plates centrifuge the deep-well plates 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.
- Set a thermal mixer or alternative heating device to +56°C.

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 buffers dispensed according to Table 10 or Table 11.

**(a)** Cartridge format:

| Well | Reagents | Volume per well |
|---|---|---|
| Position 1 | Binding Buffer | 200 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 10.** Setup for cartridge(s) compatible with Auto-Pure Mini.

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

| Well | Reagents | Volume per well |
|---|---|---|
| Column 1/7 | Binding Buffer | 200 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 11.** Setup for a V-bottom 96 deep-well plate compatible with Auto-Pure Mini.

1. For each processed sample, transfer required amount to a 1.5 ml or 2 ml microcentrifuge tube: Up to 20 hairs incl. bulbs (cut into smallest possible pieces).

2. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
3. Incubate in a thermal mixer at +56°C for 60 min with agitation at full speed (~1,400 *rpm*).
4. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 *rpm*) for 5 min.

5. Remove sealing foil from cartridge(s) or V-bottom 96 deep-well plate.
6. Transfer lysate(s) and Magnetic Beads (**orange**) to appropriate well(s) (Table 12 or Table 13).

**(a)** Cartridge format:

| Well | Sample/Reagents | Volume per well |
|---|---|---|
| Position 1 | Binding Buffer<br>**Lysate**<br>**Magnetic Beads** | 200 µl<br>**~220 µl**<br>**10 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.** Pipetting scheme for lysate and Magnetic Bead transfer into cartridge(s).

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

| Well | Sample/Reagents | Volume per well |
|---|---|---|
| Column 1/7 | Binding Buffer<br>**Lysate**<br>**Magnetic Beads** | 200 µl<br>**~220 µl**<br>**10 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.** Pipetting scheme for lysate and Magnetic Bead transfer into V-bottom 96 deep-well plate.

7. 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.
8. Set program according to Table 14.

| 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 | Bind | 1 | 500 | 5 | 7 | 0 | 37 | 2 | 30 | 0 | 1 | 3 | 80 | 0 | 0 |
| 2 | Wash 1 | 3 | 600 | 1 | 5 | 0 | 0 | 2 | 30 | 0 | 1 | 3 | 80 | 0 | 0 |
| 3 | Wash 2 | 4 | 600 | 1 | 5 | 0 | 0 | 2 | 30 | 0 | 1 | 3 | 80 | 0 | 0 |
| 4 | Wash 3 | 5 | 600 | 2 | 5 | 2.5 | 0 | 2 | 30 | 0 | 1 | 3 | 80 | 0 | 0 |
| 5 | Elution | 6 | 100 | 1 | 4 | 0 | 60 | 2 | 45 | 0 | 2 | 3 | 80 | 0 | 0 |
| 6 | Waste | 1 | 500 | 0.1 | 5 | 0 | 0 | 1 | 0 | 0 | 1 | 2.5 | 80 | 0 | 0 |

**Table 14.** Program setting for "Genomic DNA Hair Purification" on Auto-Pure Mini device.

9. Run the program.
10. 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.

## 14.4. MANUAL EXTRACTION

Preparation:

- Set a thermal mixer or alternative heating device to +37°C.
- Pre-heat appropriate volume of Elution Buffer to +60°C.

1. Transfer required amount of sample to a 1.5 ml or 2 ml microcentrifuge tube:
   Up to 20 hairs incl. bulbs (cut into smallest possible pieces).
2. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
3. Incubate in a thermal mixer at +56°C for 60 min with agitation at full speed (~1,400 rpm).
4. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm) for 5 min.
5. Vortex Magnetic Beads thoroughly. Add 200 µl Binding Buffer and 10 µl Magnetic Beads to the tube. Mix by pipetting up and down at least 5 times.
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 the 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 the beads to settle until the solution is completely clear.
12. Remove and discard the supernatant. Avoid disturbing 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 the magnetic beads to settle until the solution is completely clear.
16. Remove and discard the supernatant. Avoid disturbing 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 the 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 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. |
| | Too much protein present in starting material (excess protein may compete with gDNA for binding to Magnetic Beads) | Use less sample. |
| | 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 gDNA 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.<br>When using automated isolation, check the instrument settings and increase bead binding time, if necessary. |

**Table 15.** Troubleshooting guide.

# 16. CONSUMABLES AND RELATED PRODUCTS

| Product | Content | Cat.No. |
|---|---|---|
| SphaeraMag® Genomic DNA Hair Purification Kit - Universal 96 | 96 preps | PCCSKU16094 |
| SphaeraMag® Genomic DNA Hair Purification Kit - Pre-filled 96 | 96 preps for Phoenix-Pure 96 & Auto-Pure 96 | PCCSKU16095 |
| SphaeraMag® Genomic DNA Hair Purification Kit - Pre-filled 32 | 80 preps for Phoenix-Pure 32 & Auto-Pure 32 | PCCSKU16096 |
| SphaeraMag® Genomic DNA Hair Purification Kit - Pre-filled Mini | 48 preps for Auto-Pure Mini | PCCSKU16097 |
| 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 16.** Consumables and related products.

*Ordering information*

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

www.procomcure.com

or order via E-mail:

***sales@procomcure.com***

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ISO 9001:2015
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