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REF	Catalogue Number	LOT	Batch Code	
\bigcap i	Consult instructions for use		Manufacturer	
	Use by date	A	Temperature limit	
Σ	Contains sufficient for <n> te</n>	ests		

1. INTRODUCTION

The SphaeraMag® DNA/RNA Isolation Kit is designed for rapid and reliable isolation of pathogenic nucleic acids from saliva, nasopharyngeal swabs, nasopharyngeal aspirates, bronchoalveolar lavage samples in Universal Transport Medium (UTM)/Viral Transport Medium (VTM) and swab samples. Samples are lysed in a specifically formulated buffer to release nucleic acids which are bound to the surface of paramagnetic beads. Proteins and cellular debris are removed by washing of the beads. Nucleic acids are 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 nucleic acids can be further analyzed e.g. for diagnostic purposes (pathogen detection) or research applications (e.g. PCR analysis).

2. KIT COMPONENTS

	SphaeraMag® DNA/RNA Isolation Kit - Universal (96)	SphaeraMag [®] DNA/RNA Isolation Kit - Universal (2 x 96)	SphaeraMag® DNA/RNA Isolation Kit - Universal (10 x 96)	SphaeraMag [®] DNA/RNA Isolation Kit - Pre-filled 96	SphaeraMag [®] DNA/RNA Isolation Kit - Pre-filled 32	SphaeraMag® DNA/RNA Isolation Kit - Pre-filled Mini
Catalogue No.	PCCSKU16007	PCCSKU16020	PCCSKU16042	PCCSKU16006	PCCSKU16004	PCCSKU16072
Preps	96	192	960	96	80	48
Lysis/Binding Buffer	70 ml	2 x 70 ml	700 ml	Pre-filled	Pre-filled	Pre-filled
Wash Buffer	60 ml	2 x 60 ml	600 ml	Pre-filled	Pre-filled	Pre-filled
Elution Buffer	10 ml	2 x 10 ml	100 ml	Pre-filled	Pre-filled	Pre-filled
Magnetic Beads	1 ml	2 x 1 ml	10 x 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® DNA/RNA Isolation kit content.

3. KIT COMPATIBILITY

	Universal	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® DNA/RNA Isolation 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 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

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

7. ILLUSTRATED PROTOCOL

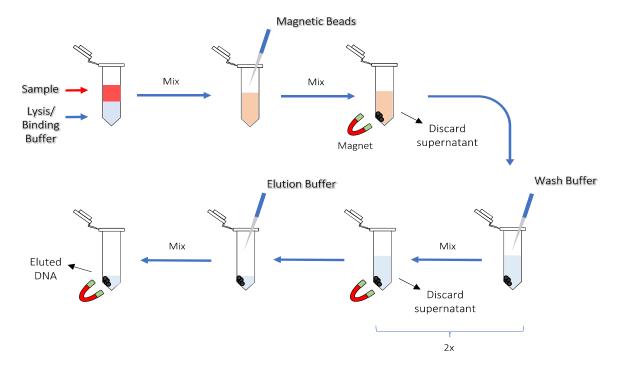


Figure 1. Illustrated protocol for manual nucleic acid isolation.

8. REQUIRED MATERIALS NOT SUPPLIED

General

- Ethanol, absolute (only for Universal)
- Suitable reagent and sample dispensing options
- Consumables for isolation devices for automated extraction
- Vortexer

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)
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)

9. SAMPLE INPUT

Recommended sample input	Saliva: up to 200 µl Nasopharyngeal aspirate: up to 200 µl Bronchoalveolar lavage sample: up to 200 µl Swab sample (in preservation solution/PBS/0.9% NaCl): 200 µl
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Table 3. Sample input.

10. 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 (only for Universal) by adding Ethanol, absolute (not supplied) as indicated on the bottle label and store at room temperature.

11. 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 +40°C to improve elution efficiency.

Volume

The recommended elution volume ranges between 50-100 μ l. A higher elution volume results in higher yield, but decreased nucleic acid concentration. A lower volume allows for higher concentration.

Storage

The eluted nucleic acids can be safely stored at +4 to +8°C for short-term storage. Regarding the type of nucleic acids (DNA or RNA) to be analyzed, the following storage conditions for short-term as well as long-term storage are strongly recommended. Repeated freeze-thaw cycles should be avoided to maintain eluate integrity.

- **DNA** should be stored at +4 to +8°C for short-term and at -80°C for long-term storage.
- RNA should be stored at -25 to -15°C for short-term and at -80°C for long-term storage.

12. EXTRACTION PROTOCOLS

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

<u>Compatible Kits:</u> SphaeraMag® DNA/RNA Isolation Kit - Universal

SphaeraMag® DNA/RNA Isolation Kit - Pre-filled 96

<u>Important:</u>

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 910 µl to avoid spilling and cross-contamination.
- 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	-
Lysis/Binding	2	Lysis/Binding Buffer	700 µl
-	3	-	-
Wash 1	4	Wash Buffer	600 µl
Wash 2	5	Wash Buffer	600 µl
Wash 3	6	Wash Buffer	600 µl
-	7	-	-
Elution	8	Elution Buffer	100 μΙ

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 Magnetic Beads (orange) to appropriate well(s) (Table 5) on Position-2 Plate (Lysis/Binding Buffer).

Step Name	Plate Position	Samples/Reagents	Volume per well
Loading	1	Tip comb	-
Lysis/Binding	2	Lysis/Binding Buffer Sample Magnetic Beads	700 μl 200 μl 10 μl
-	3	-	-
Wash 1	4	Wash Buffer	600 µI
Wash 2	5	Wash Buffer	600 µl
Wash 3	6	Wash Buffer	600 µl
-	7	-	-
Elution	8	Elution Buffer	100 μΙ

Table 5. Pipetting scheme for sample(s) and Magnetic Bead transfer into V-bottom 96 deep-well plates.

- 4. 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.
- 5. 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)	lst segment time (sec)	2 nd segment time (sec)
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Lysis/ Binding	2	5	80	0	900	3	40	2	1	3	10	10
3	Wash 1	4	1	80	0	600	5	0	2	1	3	10	10
4	Wash 2	5	1	80	0	600	5	0	2	1	3	5	5
5	Wash 3	6	1	80	2.5	600	5	0	2	1	3	5	5
6	Elution	8	5	80	0	100	3	40	1	3	3	30	1
7	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 6. Program setting for "DNA/RNA Isolation" on Phoenix-Pure 96/Auto-Pure 96 device.

- 6. Run the program.
- 7. 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 for long-term or short-term according to recommended storage conditions (see section 11).

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

<u>Compatible Kits:</u> SphaeraMag® DNA/RNA Isolation Kit - Universal SphaeraMag® DNA/RNA Isolation 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 910 µl to avoid spilling and cross-contamination.
- 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	Lysis/Binding Buffer	700 µl
Column 2/8	-	-
Column 3/9	Wash Buffer	600 µI
Column 4/10	Wash Buffer	600 µI
Column 5/11	Wash Buffer	600 µl
Column 6/12	Elution Buffer	100 μΙ

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 plates.
- 3. Transfer sample(s) and Magnetic Beads (orange) to appropriate well(s) in columns 1/7 (Table 8).

Well	Samples/Reagents	Volume per well
Column 1/7	Lysis/Binding Buffer Sample Magnetic Beads	700 µl 200 µl 1 0 µl
Column 2/8	-	-
Column 3/9	Wash Buffer	600 µl
Column 4/10	Wash Buffer	600 µl
Column 5/11	Wash Buffer	600 µl
Column 6/12	Elution Buffer	100 μΙ

Table 8. Pipetting scheme for sample(s) and Magnetic Bead transfer into an U-bottom 96 deep-well plate.

- 1. Load an U-bottom 96 deep-well plate(s) onto the instrument.

 Note: Make sure that plates are placed in the correct orientation.
- 2. Place new clean tip comb(s) in the instrument.
- 3. Set program according to Table 9.

step	step name	ew	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/ Binding	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	100	3	40	0	80	0	3
6	Waste	1	0.2	0	0	900	3	0	0	80	0	3

Table 9. Program setting for "DNA/RNA Isolation" on Phoenix-Pure 32/Auto-Pure 32 device.

- 4. Run the program.
- 5. 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 for long-term or short-term according to recommended storage conditions (see section 11).

12.3. AUTOMATED PURIFICATION: AUTO-PURE MINI

<u>Compatible Kit:</u>
SphaeraMag® DNA/RNA Isolation Kit - Universal
SphaeraMag® DNA/RNA Isolation 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 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 910 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 buffers dispensed according to Table 10 or Table 11.

(a) Cartridge format:

Well	Reagents	Volume per well
Position 1	Lysis/Binding Buffer	700 µl
Position 2	-	-
Position 3	Wash Buffer	600 µl
Position 4	Wash Buffer	600 µl
Position 5	Wash Buffer	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	Lysis/Binding Buffer	700 µl
Column 2/8	-	-
Column 3/9	Wash Buffer	600 µI
Column 4/10	Wash Buffer	600 µI
Column 5/11	Wash Buffer	600 µl
Column 6/12	Elution Buffer	100 μΙ

Table 11. Setup for a V-bottom 96 deep-well plate compatible with Auto-Pure Mini.

- 1. Remove sealing foil from cartridge(s) or from the V-bottom 96 deep-well plate.
- 2. Transfer sample(s) and Magnetic Beads (orange) to appropriate well(s) (Table 12 or Table 13).

(a) Cartridge format:

Well	Samples/Reagents	Volume per well
Position 1	Lysis/Binding Buffer Sample Magnetic Beads	700 µl 200 µl 10 µl
Position 2	-	-
Position 3	Wash Buffer	600 µl
Position 4	Wash Buffer	600 µl
Position 5	Wash Buffer	600 µl
Position 6	Elution Buffer	100 µl

Table 12. Pipetting scheme for sample and Magnetic Bead transfer 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 10 µl
Column 2/8	-	-
Column 3/9	Wash Buffer	600 µI
Column 4/10	Wash Buffer	600 μΙ
Column 5/11	Wash Buffer	600 μΙ
Column 6/12	Elution Buffer	100 μΙ

Table 13. Pipetting scheme for sample and Magnetic Bead transfer into the V-bottom 96 deep-well plate.

- 3. Load cartridge(s) or the V-bottom 96 deep-well plate onto instrument. Note: Make sure that cartridge(s) or the -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 14.

step	step name	hole site	volume (µl)	mix fime (min)	mix speed	dry time (min)	temperature (°C)	segments	every time (sec)	magnetization time (s)	cycle	magnet speed (mm/sec)	mix scope (%)	Mix pos.	Magnet pios.
1	Lysis/ Binding	1	900	5	3	0	40	2	30	0	1	3	80	0	0
2	Wash 1	3	600	1	5	0	_	2	30	0	1	3	80	0	0
3	Wash 2	4	600	1	5	0	-	2	30	0	1	3	80	0	0
4	Wash 3	5	600	1	5	2.5	-	2	30	0	1	3	80	0	0
5	Elution	6	100	5	3	0	40	2	45	0	1	3	80	0	0
6	Waste	1	900	0.1	5	-	-	1	0	0	0	2.5	80	0	0

Table 14. Program setting for "DNA/RNA Isolation" on Auto-Pure Mini device.

- 6. Run the program.
- 7. After the run is finished, remove cartridge(s) or 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 for long-term or short-term according to recommended storage conditions (see section 11).

12.4. MANUAL EXTRACTION

Preparation:

- Pre-heat appropriate volume of Lysis/Binding Buffer to +40°C.
- Pre-heat appropriate volume of Elution Buffer to +40°C.
- 1. Transfer 700 µl Lysis/Binding Buffer to a 1.5 ml or 2 ml microcentrifuge tube.
- 2. Add max. 200 µl sample to the tube. Mix by pipetting up and down at least 5 times. Note: If processing less than 200 µl sample, bring volume up to 200 µl with nuclease-free water.
- 3. Vortex Magnetic Beads thoroughly. Add 10 µl Magnetic Beads to the tube. Mix by pipetting up and down at least 10 times or by shaking for 1 min. Incubate at room temperature for 2 min.
- 4. Place the tube on a magnetic stand. Allow the magnetic beads to settle until the solution is completely clear.
- 5. Remove and discard the supernatant. Avoid disturbing the magnetic bead pellet.
- 6. Add 600 µl Wash Buffer.
- 7. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
- 8. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
- 9. Remove and discard the supernatant. Avoid disturbing magnetic bead pellet.
- 10. Repeat steps 6-9 for an additional washing step with Wash Buffer.
- 11. Remove any residual liquid. Leave the tube open for 5 min to allow magnetic beads air dry.
- 12. Add 100 µl pre-heated Elution Buffer to the tube.
- 13. Remove the tube from the magnetic stand. Mix well by pipetting up and down ~10 times.
- 14. Incubate at room temperature for 1 min.
- 15. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
- 16. Transfer the eluate to a clean tube. Process directly or store for long-term or short-term according to recommended storage conditions (see section 11).

13. 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.

Problem	Possible Cause	Solution			
	Incomplete resuspension of SphaeraMag® Magnetic Beads	Thoroughly resuspend SphaeraMag® Magnetic Beads before use			
	DNA/RNA degradation during sample handling or storage	Process sample immediately after collection or removal from adequate storage.			
Low Yield	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 DNA/RNA for binding to Magnetic Beads)	Use less sample.			
	Insufficient sample material	Increase lysis and binding time if the sample is diluted.			
	Poor DNA/RNA quality	Do not freeze/thaw the isolated DNA/RNA more than once or store at room temperature.			
Problems with downstream applications	Insufficient DNA/RNA was used	Quantify the purified DNA/RNA accurately and use sufficient DNA/RNA.			
	Ethanol carry-over	Dry the SphaeraMag® Magnetic Beads completely before adding Elution Buffer.			
Carry-over of Magnetic Beads	SphaeraMag® Magnetic Beads did not fully magnetize on last step	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 15. Troubleshooting guide.

14. CONSUMABLES AND RELATED PRODUCTS

	Content	Cat.No.	
	96 preps	PCCSKU16007	
SphaeraMag® DNA/RNA Isolation Kit - Universal	2 x 96 preps	PCCSKU16020	
	10 x 96 preps	PCCSKU16042	
SphaeraMag® DNA/RNA Isolation Kit - Pre-filled 32	80 preps for Phoenix-Pure-32 & Auto-Pure-32	PCCSKU16004	
SphaeraMag® DNA/RNA Isolation Kit - Pre-filled 96	96 preps for Phoenix-Pure-96 & Auto-Pure-96	PCCSKU16006	
SphaeraMag® DNA/RNA Isolation Kit- Pre-filled Mini	48 preps for Auto-Pure-Mini	PCCSKU16072	
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. Consumbales and related products.

Ordering information

For ordering SphaeraMag® DNA/RNA Isolation Kit and other products, visit us at

www.procomcure.com

or order via E-mail:

sales@procomcure.com



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