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SphaeraMag® DNA/RNA Isolation

Nucleic Acid Extraction for Manual and Automated Systems

INTRODUCTION

The SphaeraMag® DNA/RNA Isolation Kit is designed for rapid and reliable isolation of total nucleic acids from whole blood, serum, plasma, saliva, nasopharyngeal swabs, nasopharyngeal aspirates, bronchoalveolar lavage samples in Universal Transport Medium (UTM)/ Viral Transport Medium (VTM) and swab samples. Isolated nucleic acids can then be further analysed e.g. for diagnostic purposes (pathogen detection) or research applications (e.g. PCR analysis).

Samples are lysed in a specially formulated buffer to release the nucleic acids which are then bound to the surface of paramagnetic beads. Proteins and cellular debris are removed by washing of the beads. The purified RNA is then eluted in the provided elution buffer, nuclease-free water or other low ionic strength buffer. The procedure can be scaled up or down and performed manually or with an automated system like Phoenix-Pure.

When using the SphaeraMag® DNA/RNA Isolation Kit for the first time, please read this booklet before starting to become familiar with the procedure.

	SphaeraMag® Universal 96 preps	SphaeraMag® pre-packed32 80 preps	SphaeraMag® pre-packed96 96 Preps
Lysis/Binding Buffer	60 ml	pre-packed	pre-packed
Wash Buffer	180 ml	pre-packed	pre-packed
SphaeraMag® Magnetic Beads	1 ml	1 ml	1 ml
Carrier RNA	2 vials	2 vials	2 vials
Elution Buffer	25 ml	pre-packed	pre-packed

Lysis/Binding Buffer and Wash Buffer contain chaotropic salts which are irritants. Please handle with appropriate laboratory safety measures and wear gloves.

STORAGE AND STABILITY

The expiry date of the kit is stated on the outer packaging and the reagents itself. Store reagents as follows:

- SphaeraMag® Magnetic Beads must be stored at 2-8°C.
- Carrier RNA should be stored at -20°C after resuspension.
- All remaining components should be stored at room temperature.

ADDITIONAL MATERIALS REQUIRED

- Ethanol, absolute (only for SphaeraMag® Universal)
- Consumables for Phoenix-Pure isolation devices for automated extraction
- Magnetic separation rack for manual isolation
- Suitable reagent dispensing options (e.g. liquid handling system, multichannel pipettes)

PREPARING REAGENTS

- Dilute Wash Buffer with 100% ethanol as stated on the bottle (only for SphaeraMag® Universal), store at room temperature.
- Add 250 µl nuclease-free water or Elution Buffer to the tube containing lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots and store it at –20°C. Do not freeze–thaw the aliquots of Carrier RNA more than 3 times.
- Sterilize the instrument by UV light before use.
- Lysis Buffer and Wash Buffer may form precipitates when stored under cool conditions. Check buffers for precipitate before use and re-dissolve at 37°C if necessary.
- For automated isolation, distribute the reagents according to Table 1.

It is recommended to inactivate viruses before DNA/RNA isolation.

SAMPLE PREPARATION

Swab

For swabs with preservation solution, transfer max. 250 µl supernatant to the plate for automatic isolation.

For swabs without preservation solution, add 500-600 µl PBS / 0.9% NaCl solution to the sample, vortex and incubate for 10 min. Transfer max. 250 µl supernatant to the plate for automatic isolation.

Broncheoalveolar lavage and Sputum

Add max. 250 µl sample to the plate for automatic isolation.

AUTOMATION STEPS FOR PHOENIX-PURE32

1. Take a 96-deepwell plate and add samples and reagents to the plate according to Table 1. The total volume of each well should not exceed 900 µl to avoid spilling and cross-contamination.

Table 1. Setting of 96-well plate and reagent dosage

Well	Samples/Reagents	Volume (µl)
Column 1/7	Lysis/Binding Buffer Carrier RNA Samples SphaeraMag® Magnetic Beads	600 µl Lysis / Binding Buffer 5 µl Carrier RNA max. 250 µl sample 10 µl SphaeraMag® Magnetic Beads
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	200 µl

2. Start the instrument and set the program according to Table 2.

Table 2. Program Setting of Phoenix-Pure32

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	8	60	0	900	8	/	0	80	0	1
2	wash 1	3	1	60	0	600	8	/	0	80	0	1
3	wash 2	4	1	45	0	600	8	/	0	80	0	1
4	wash 3	5	1	45	2.5	600	8	/	0	80	0	1
5	elute	6	5	90	0	200	8	/	0	80	0	1
6	drop	5	0.2	0	0	600	5	/	0	80	0	1

3. Place a new clean magnetic rods tip in the instrument (Re-Use of magnetic rods tip will cause cross-contamination). Place the 96-well plate containing the sample and reagent into the instrument, corresponding to the magnetic rod. Remember to place the magnetic rods tip or the reagent will corrode the magnetic bar.

4. Execute program.

5. Collect DNA/RNA: Remove the 96-well plate and magnetic rods tip. Transfer the cleared supernatant of Column 6/12 into a new tube if required, store at -20°C or -80°C or process directly.

AUTOMATION STEPS FOR PHOENIX-PURE96

1. Take 6 pieces of 96-well plates and add samples and reagents according to Table 3. The total volume of each well should not exceed 900 µl to avoid spilling and cross-contamination.

Table 3. Setting of 96-well plate and reagent dosage

Step	Plate Position	Samples/Reagents	Volume (µl)
Loading	1	Tip Comb	/
Binding	2	Lysis/Binding Buffer Carrier RNA Samples SphaeraMag® Magnetic Beads	600 µl Lysis / Binding Buffer 5 µl Carrier RNA max. 250 µl sample 10 µl SphaeraMag® Magnetic Beads
Wash 1	4	Wash Buffer	600 µl
Wash 2	5	Wash Buffer	600 µl
Wash 3	6	Wash Buffer	600 µl
Elution	8	Elution Buffer	200 µl

2. Start the instrument, place the 6 plates and the tip comb on the corresponding position in the instrument. Make sure that A1 of the plate is at the A1 position of the instrument.

3. Set the program according to Table 4.

Table 4. Program Setting of Phoenix-Pure96

step	step name	plate	mix time (min)	mix amp (%)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	segment (1-5)	cycle time (1-10)	Magnet speed (1-10)	1st segment time	2nd segment time
1	Load	1											
2	Binding 1	2	8	80	0	900	3	OFF	2	1	3	10	10
3	Wash 1	4	1	80	0	600	3	OFF	2	1	3	10	10
4	Wash 2	5	1	80	0	600	3	OFF	2	1	3	5	5
5	Wash 3	6	1	80	2.5	600	3	OFF	2	1	3	5	5
6	Elution	8	5	80	0	200	3	OFF	1	3	3	30	
7	Unload	1											

4. Execute program.

5. Collect DNA/RNA: Remove the 96-well plates and magnetic rods tip. Transfer the cleared supernatant of the elution plate into new tubes if required, store at -20°C or -80°C or process directly.

MANUAL DNA/RNA ISOLATION PROTOCOL

1. Freshly prepare the following lysis mastermix per sample.
   Lysis Buffer 600 µl
   Carrier RNA 5 µl

2. Transfer 605 µl lysis mastermix to each 1.5 ml / 2 ml vial.

3. Add max. 250 µl of sample into each vial. Mix by shaking for 1 minute. If using frozen samples, thaw at room temperature and mix well by shaking or pipetting up and down before proceeding to Step 4.
Note: If the sample is less than 250 µl, bring the volume up to 250 µl with nuclease-free water.

4. Add 10 µl SphaeraMag® Magnetic Beads to each tube. Mix by shaking for 5 minutes.

5. Place the vials on a magnetic separation device to magnetize SphaeraMag® Magnetic Beads. Let sit at room temperature until the SphaeraMag® Magnetic Beads are completely cleared from solution.

6. Aspirate and discard the supernatant. Do not disturb the SphaeraMag® Magnetic Beads.

7. Remove the vials from the magnetic separation device.

8. Add 600 µl Wash Buffer to each tube.
Note: Wash Buffer must be diluted with ethanol prior to use.

9. Resuspend the SphaeraMag® Magnetic Beads by shaking for 1 minute.
Note: Complete resuspension is required for adequate washing of the SphaeraMag® Magnetic Beads.

10. Place the vials on the magnetic separation device to magnetize the SphaeraMag® Magnetic Beads. Let sit at room temperature until the SphaeraMag® Magnetic Beads are completely cleared from solution.

11. Aspirate and discard the supernatant. Do not disturb the SphaeraMag® Magnetic Beads.

12. Remove the vials from the magnetic separation device.

13. Repeat Steps 8-11 for a second washing step.

14. Leave the vials on the magnetic separation device for 10 minutes to air dry the SphaeraMag® Magnetic Beads. Remove any residual liquid with a pipettor.

15. Remove the vials from the magnetic separation device.

16. Add 100 µl Elution Buffer to each tube.

17. Resuspend the SphaeraMag® Magnetic Beads by shaking for 2 minutes.

18. Let sit at room temperature for 5 minutes. Place the vials on the magnetic separation device to magnetize SphaeraMag® Magnetic Beads. Let sit at room temperature until the SphaeraMag® Magnetic Beads are completely cleared from solution.

19. Transfer the cleared supernatant containing purified DNA/RNA to a clean tube. Store at -70°C or process directly

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
Low Yield	Incomplete resuspension of magnetic beads	Thoroughly resuspend SphaeraMag® Magnetic Beads before use
	DNA/RNA degraded during sample handling /storage	Immediately process sample after collection or removal from storage.
	Wash Buffer not prepared correctly	Prepare Wash Buffer with the correct amount of ethanol
	Insufficient sample material	Increase binding time if the sample is diluted.
Problems with downstream applications	Poor DNA/RNA quality	Do not freeze / thaw the isolated DNA/ RNA more than once or store at room temperature. Check isolate for degradation.
	Insufficient 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 separation device for an additional 5 minutes or centrifuge at >4,000 x g for 5 minutes. When using automated isolation, check instrument settings and increase bead binding time if necessary.

Consumables & Related Products

	Content	Cat.No.
SphaeraMag® DNA/RNA Isolation Kit 96-Universal	96 preps	PCCSKU16007
SphaeraMag® pre-packed32	80 preps for Phoenix-Pure-32 & Auto-Pure-32	PCCSKU16004
SphaeraMag® pre-packed96	96 preps for Phoenix-Pure-96 & Auto-Pure-96	PCCSKU16006
Tip Combs for AutoPure-96 & Phoenix-Pure-96	8 pcs	PCCSKU16012
Magnetic Rods for AutoPure-32 & Phoenix-Pure-32	20 Pcs	PCCSKU16013
U-Bottom Plates for AutoPure-32 & Phoenix-Pure-32	20 deepwell plates	PCCSKU16014
V-Bottom Plates for AutoPure 96 & Phoenix-Pure-96	5 deepwell plates	PCCSKU16015
	50 deepwell plates	PCCSKU16016

Ordering Information

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

www.procomcure.com/shop

or order via E-mail:

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