trax LAB SYSTEMS

SphaeraMag® DNA/RNA Isolation Kit

Nucleic Acid Extraction for Manual and Automated Systems

Instruction Manual

Version 3.2

INTRODUCTION

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

Samples are lysed in a specifically 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 nucleic acids are then eluted in the provided elution buffer, nuclease-free water or other low ionic strength buffer. The procedure can be performed manually or by using automated systems (like Auto-Pure).

	SphaeraMag® DNA/RNA Isolation Kit - Universal	SphaeraMag® DNA/RNA Isolation Kit - Universal	SphaeraMag® DNA/RNA Isolation Kit - Universal	SphaeraMag® DNA/RNA Isolation Kit - pre-filled 32	SphaeraMag® DNA/RNA Isolation Kit - pre-filled 96	SphaeraMag® DNA/RNA Isolation Kit - pre-filled Mini
Preps	96	2 x 96	1 x 960	80	96	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
SphaeraMag® Magnetic Beads	1 ml	2 x 1 ml	10 x 1 ml	1 ml	1 ml	1 ml
Elution Buffer	10 ml	2 x 10 ml	100 ml	pre-filled	pre-filled	pre-filled

Table 1. Content of SphaeraMag® DNA/RNA Isolation kit.

Lysis/Binding Buffer contains chaotropic salts. Please handle with appropriate laboratory safety measures.

STORAGE AND STABILITY

- Upon arrival, SphaeraMag® Magnetic Beads must be stored at +2 °C to +8 °C.
- All other kit components can be stored at room temperature (+15 °C to +25°C).
- The expiry dates of the kit and single components are stated on the outer packaging and each individual reagent.

ADDITIONAL MATERIALS REQUIRED

- Ethanol, absolute (SphaeraMag® Universal)
- Suitable reagent dispensing options
- Consumables for isolation devices for automated extraction
- Magnetic separation rack for manual isolation

PRE-APPLICATION PREPARATION

- Dilute Wash Buffer with absolute ethanol as stated on the bottle (only for SphaeraMag® Universal). Store at room temperature.
- For automated isolation, distribute the reagents into appropriate plate according to respective protocol used.

KIT COMPATIBILITY

- Hamilton Microlab® STAR / MagEx STARlet / NIMBUS
- KingFisher™ BioSprint®
- Tecan Dream Prep™ NAP
- Phoenix-Pure-96 / Phoenix-Pure-32
- Allsheng Auto-Pure-96 / Auto-Pure-32 / Auto-Pure Mini

SAMPLE PREPARATION

Swab
For swabs with preservation solution, process max. 200 µl supernatant. For swabs without preservation solution, add 500- 600 µl PBS / 0.9% NaCl solution to the sample, vortex and incubate for 10 min. Then, process max. 200 µl of the supernatant.

Broncheoalveolar lavage and sputum
Process max. 200 µl sample.

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

AUTOMATED PURIFICATION: AUTO-PURE-96 / PHOENIX-PURE-96

Compatible Kits: SphaeraMag® DNA/RNA Isolation Kit - Universal
SphaeraMag® DNA/RNA Isolation Kit - pre-filled 96

Important:

- Resuspend SphaeraMag® Magnetic Beads thoroughly for 30 sec before use.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Take six V-bottom 96 deep-well plates. When using pre-filled plates, carefully remove the sealing foil. Add samples and SphaeraMag® Magnetic Beads to appropriate wells according to Table 2.

Step	Plate Position	Samples/Reagents	Volume
Loading	1	Tip comb	/
Binding	2	Lysis/Binding Buffer **Sample** **SphaeraMag® Magnetic Beads**	700 µl **max. 200 µl** **10 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 µl

Table 2. Setup of 96 deep-well plates and reagent volumes compatible with Auto-Pure-96.

2. Start the instrument. Place the deep-well plates on the corresponding positions in the instrument. Note: Make sure that all plates are placed in the correct orientation.

3. Set the program according to Table 3.

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	0	1	0	5	1	0	1	1	1	1	1
2	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 3. Program setting for "SphaeraMag® DNA/RNA Isolation" on Auto-Pure-96 device.

4. Run program.

5. After the run is finished, remove plates and tip comb. Transfer the eluate from the elution plate to new tubes if required. Process directly or store at -20 °C, preferably -80 °C for long term storage.

AUTOMATED PURIFICATION: AUTO-PURE-32 / PHOENIX-PURE-32

Compatible Kit: SphaeraMag® DNA/RNA Isolation Kit - pre-filled 32

Important:

- Resuspend SphaeraMag® Magnetic Beads thoroughly for 30 sec before use.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Take an U-bottom 96 deep-well plate. When using pre-filled plates, carefully remove the sealing foil. Add samples and SphaeraMag® Magnetic Beads to appropriate wells according to Table 4.

Well	Samples/Reagents	Volume
Column 1/7	Lysis/Binding Buffer **Sample** **SphaeraMag® Magnetic Beads**	700 µl **max. 200 µl** **10 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 µl

Table 4. Setup of 96 deep-well plate and reagent volumes compatible with Auto-Pure-32.

2. Start the instrument. Place new clean tip comb(s) in the instrument (re-use of tip combs will cause cross-contamination). Place the 96-well plate containing sample(s) and reagents into the instrument.

3. Set the program according to Table 5.

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	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 5. Program setting for "SphaeraMag® DNA/RNA Isolation" on Auto-Pure-32 device.

4. Run program.

5. After the run is finished, remove deep-well plate and tip comb(s). Transfer the eluate from column(s) 6/12 to new tubes if required. Process directly or store at -20 °C, preferably -80 °C for long term storage.

AUTOMATED PURIFICATION: AUTO-PURE MINI

Compatible Kit: SphaeraMag® DNA/RNA Isolation Kit - pre-filled Mini

Important:

- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Take required amount of extraction cartridges (1 per sample). Carefully remove the sealing foil. Add sample to appropriate well according to Table 6.

Well	Samples/Reagents	Volume
Position 1	Lysis/Binding Buffer **Sample**	700 µl **max. 200 µl**
Position 2	-	-
Position 3	SphaeraMag® Magnetic Beads	320 µl
Position 4	Wash Buffer	600 µl
Position 5	Wash Buffer	600 µl
Position 6	Elution Buffer	100 µl

Table 6. Setup of extraction cartridge and reagent volumes compatible with Auto-Pure Mini.

2. Start the instrument. Place new clean tip comb(s) in the instrument (re-use of tip combs will cause cross-contamination). Place extraction cartridge containing the sample and reagent into the instrument.

3. Set the program according to Table 7.

step	step name	hole site	volume (µl)	mix time (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	Bead	3	320	1	8	0	-	2	30	0	1	1	80	0	0
2	Lysis	1	900	5	3	0	40	2	30	0	1	3	80	0	0
3	Wash 1	4	600	1	5	0	-	2	30	0	1	3	80	0	0
4	Wash 2	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 7. Program setting for "SphaeraMag® DNA/RNA Isolation" on Auto-Pure Mini device.

4. Run program.

5. After the run is finished, remove extraction cartridge(s) and tip comb(s). Transfer the eluate from position 6 to a new tube if required. Process directly or store at -20°C, preferablyy -80 °C for long term storage.

MANUAL PURIFICATION

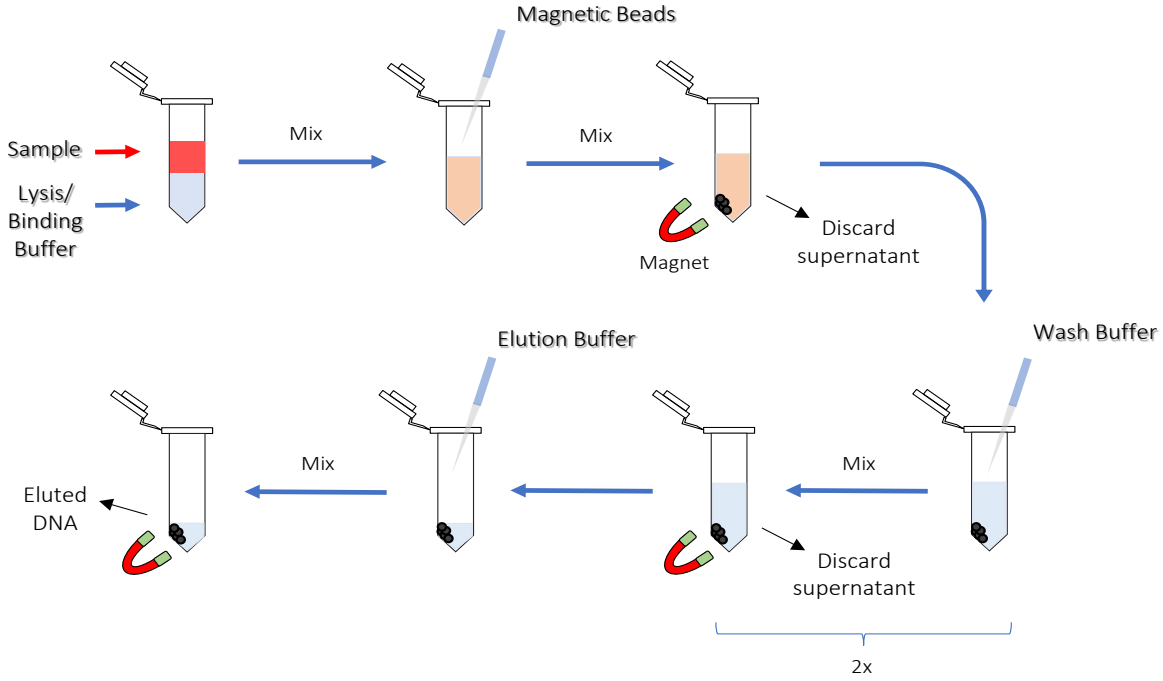

Figure 1. Illustrated purification protocol.

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 clean 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. Thorougly resuspend Magnetic Beads by vigorous vortexing for 30 sec. Add 10 µl Magnetic Beads to each tube. Mix well by pipetting several times up and down or by shaking for 1 min.
4. Place tube on magnetic stand. Let sit until Magnetic Beads are completely separated from solution.
5. Remove and discard supernatant. Avoid disturbing Magnetic Bead pellet.
6. Remove tube from magnetic stand.
7. Add 600 µl Wash buffer. Mix well by pipetting several times up and down.
8. Place tube on magnetic stand. Let sit until Magnetic Beads are completely separated from solution.
9. Remove and discard supernatant. Avoid disturbing Magnetic Bead pellet.
10. Repeat steps 7-9 for a second washing step.
11. Leave tube open allowing Magnetic Beads to air dry for 10 min. Remove any residual liquid.
12. Remove tube from magnetic stand.
13. Add 100 µl Elution buffer to the tube. Mix well by pipetting up and down at least 10 times.
14. Incubate at room temperature for 5 min.
15. Place tube on magnetic stand. Let sit until Magnetic Beads are completely separated from solution.
16. Transfer clear supernatant containing eluate to a clean tube. Process directly or store at -20 °C, preferably -80 °C for long term storage.

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 SphaeraMag® 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.

Table 8. Troubleshooting Guide.

CONSUMABLES & RELATED PRODUCTS

	Content	Cat.No.
SphaeraMag® DNA/RNA Isolation Kit - Universal	96 preps	TRAXSKU16007
	2 x 96 preps	TRAXSKU16020
	10 x 96 preps	TRAXSKU16042
SphaeraMag® DNA/RNA Isolation Kit - pre-filled 32	80 preps for Phoenix-Pure-32 & Auto-Pure-32	TRAXSKU16004
SphaeraMag® DNA/RNA Isolation Kit - pre-filled 96	96 preps for Phoenix-Pure-96 & Auto-Pure-96	TRAXSKU16006
SphaeraMag® DNA/RNA Isolation Kit- pre-filled Mini	48 preps for Auto-Pure-Mini	TRAXSKU16072
Tip Combs for AutoPure-96 & Phoenix-Pure-96	60 pcs	TRAXSKU30014
Magnetic Rods for AutoPure-32 & Phoenix-Pure-32	20 pcs	TRAXSKU16013
U-Bottom Plates for AutoPure-32 & Phoenix-Pure-32	20 deepwell plates	TRAXSKU16014
V-Bottom Plates for AutoPure 96, Phoenix-Pure-96 & AutoPure Mini	50 deepwell plates	TRAXSKU30002

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Ordering information

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

at www.traxlabsystems.com

or order via E-mail:

sales@traxconnects.com

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