EXSIG

Primerdesign™ Ltd

exsig™ Mag

5000 extractions

For Research Use Only. Not for use in diagnostic procedures.

Z-exsig™ Mag

Part of the

NOVACYT GROUP

EXSIG

Workflow Solutions by Primerdesign

Contents

1. – Introduction 2
2. – Kit contents and storage conditions 2
3. – Experimental Procedure 2
3.1 – General Information before starting 2
3.2 – Required Materials 3
3.3 – Initial Preparations 4
3.4 – Overview of the exsig™ Mag purification protocol 4
3.5 – Step-by-step protocol for nucleic acid purification 4
4.0 – Automating the nucleic acid purification protocol 5
4.1 – Automation on the KingFisher Flex 5
5.0 – Troubleshooting 6
5.1 – Common troubleshooting solutions 6
5.2 – Frequently asked questions (FAQs) 7
6.0 – Safety Information 7
7.0 – Further Support 9

exsig™ Mag Handbook

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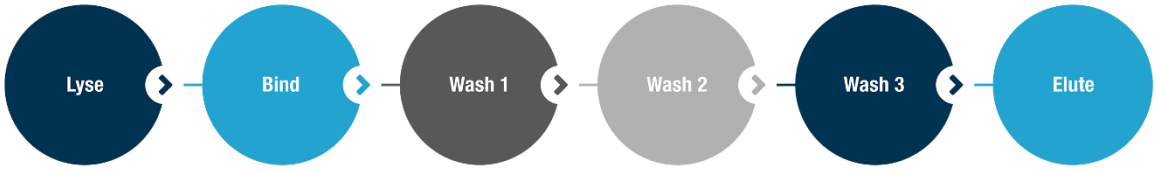
1. – Introduction

exsig™ Mag from Primerdesign™ Ltd uses magnetic separation for the purification of nucleic acid, including viral RNA from nasopharyngeal swab and sputum samples. Superparamagnetic particles coated with exsig™ Mag surface chemistry use a novel binding mechanism which, when combined with the washing steps, removes impurities present in the sample matrix. After washing, the RNA is eluted and is ready for use in downstream real-time PCR applications.



This kit is validated for research use only. It is not intended for use in diagnostic procedures.

2. – Kit contents and storage conditions

All kit components should be used by the expiry date stated on the kit box and stored under the recommended storage conditions.

Component	Colour	Volume	Storage
exsig™ Mag lysis buffer	Blue	1 x 600mL	Room temperature
exsig™ Mag binding buffer	Green	1 x 1000mL	Room temperature
exsig™ Mag particle suspension	White	1 x 110mL	Room temperature; 4 °C after opening
Wash buffer exsig™ Mag1	Red	1 x 2200mL	Room temperature
Wash buffer exsig™ Mag2	Red	1 x 2200mL	Room temperature
Wash buffer exsig™ Mag3	Yellow	1 x 600mL	Room temperature; 4 °C after opening
exsig™ Mag elution buffer	Black	1 x 600mL	Room temperature; 4 °C after opening

Table 1: exsig™ Mag kit components and storage conditions.

3. – Experimental Procedure

3.1– General Information before starting

When performing the exsig™ Mag RNA purification protocol, a magnetic rack or centrifuge is required to pellet the magnetic particles.

If performing the protocol manually without access to a magnetic rack, sample tubes can be centrifuged for 10 seconds (single tubes: full speed; plates: 2000 x g) to enable the magnetic particles to form a pellet.

All processes should be carried out at room temperature (15-25 °C) unless otherwise stated.

It is important to ensure that you have properly resuspended the exsig™ Mag particle suspension before adding to the exsig™ Mag binding buffer. Use of non-homogenous exsig™ Mag beads will affect the efficiency of the purification chemistry, potentially resulting in lower yields.

The following considerations (Table 2) should be applied to the experimental process, each time the specific protocol process is stated in the step-by-step method:

Protocol process	Consideration
Bring magnetic rack into contact with tubes	This will allow the exsig™ Mag beads to form a pellet on the side of the tube, to allow for easy removal of the supernatant. The times stated for exsig™ Mag bead pelleting are minimum recommended incubation times. The strength of the magnetic rack will influence the speed of exsig™ Mag beads pelleting. If required, increasing incubation time should be used to ensure all beads are pelleted.
Mix thoroughly	The sample should be mixed thoroughly (preferably using a shaker), to ensure the exsig™ Mag beads are completely resuspended. The mixing can be assisted by pulse vortexing in 5-10 second bursts.
Removal of supernatant	When removing supernatant, it is important to remove as much liquid as possible without dislodging the particle pellet. To avoid disruption of the particle pellet when placing the pipette tip inside the tube, ensure that the tip is aimed towards the sample tube wall opposite the pellet. It is recommended to aspirate once, let any liquid run down the walls of the tube, and then aspirate a second time to remove any remnants of liquid.
Constant shaking	The sample should be constantly agitated by vortexing/shaking to ensure the exsig™ Mag beads do not settle. This movement will increase the efficiency of the binding and washing steps.

Table 2: Technical descriptions of processes required in this protocol, and considerations that should be adhered to when performing these steps.

3.2– Required Materials

a. Magnetic rack or centrifuge
b. 96- or 384-well plates or reaction tubes that are RNase-free
c. Water bath or incubator (for temperatures up to 55 °C)

d. Optional: carrier molecule
e. Optional: protease solution

3.3– Initial Preparations

a. Presence of precipitates: Salt precipitates can form in the buffers at low temperatures. Check for the presence of precipitates prior to use, and if required, incubate buffers at 37 °C until the precipitates have re-dissolved.
b. Preparing the exsig™ Mag particle suspension: The exsig™ Mag particle suspension and exsig™ Mag binding buffer can be added to the reaction(s) as a premix. To prepare the premix for the exsig™ Mag protocol:
    i. Thoroughly mix the exsig™ Mag particle suspension to fully resuspend the particles.
    ii. Add 20 µL exsig™ Mag particle suspension to 160 µL exsig™ Mag binding buffer.
    iii. If preparing premix for multiple reactions, multiply the volumes accordingly and allow sufficient overage for accurate pipetting.

3.4– Overview of the exsig™ Mag purification protocol

Table 3 below summarises the standard manual exsig™ Mag protocol, including volumes of each component and the time and temperature for each step.

STEP	Lysis				Binding	Wash	Elution
COMPONENT	Optional: Protease solution (20 µL)	Optional: Carrier molecule	Sample (100 µL)	exsig™ Mag lysis buffer (100 µL)	exsig™ Mag binding buffer (160 µL) + exsig™ Mag particle suspension (20 µL)	exsig™ Mag wash buffers 1. (400 µL) 2. (400 µL) 3. (400 µL)	exsig™ Mag elution buffer (100 µL)
CONDITION				3 min 55 °C	10 min Room temp	5 min Room temp	10 min 60 °C

Table 3. Summary of the standard manual exsig™ Mag protocol.

3.5– Step-by-step protocol for nucleic acid purification

1. Add the following to the reaction tube in the order listed below:
    a. Optional: 20 µL Proteinase solution to the reaction tube/well
    b. Optional: 1 µg carrier molecule
    c. 100 µL of the liquid starting sample (see section 1)
    d. 100 µL (1x) exsig™ Mag lysis buffer.
2. Incubate at 55 °C for 3 minutes with constant shaking.
3. Allow the sample(s) to cool to room temperature.

4. Add 20 µL exsig™ Mag particle suspension and 160 µL exsig™ Mag binding buffer (these can be added as a 180 µL of premix – see section 2).
5. Mix thoroughly and incubate for 5 minutes at room temperature with, constant shaking.
6. Bring magnet into contact with the tube(s) for 2 minutes.
7. Remove the supernatant and discard.
8. Separate the magnet from the sample tube(s).
9. Add 400 µL exsig™ Mag wash buffer 1.
10. Incubate for 1 minute at room temperature, with constant shaking.
11. Bring magnet into contact with the tube(s) for 2 minutes.
12. Remove the supernatant and discard.
13. Separate the magnet from the sample tube(s).
14. Repeat steps 9-13 with exsig™ Mag wash buffer 2.
15. Repeat steps 9-13 with exsig™ Mag wash buffer 3.
16. Add 100 µL exsig™ Mag elution buffer. Mix thoroughly.
17. Incubate for 5 minutes at 60 °C with periodic shaking.
18. Bring magnet into contact with the tube(s) for 3 minutes.
19. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any exsig™ Mag beads.

4.0 – Automating the nucleic acid purification protocol

After trialling the exsig™ Mag protocol for your sample type manually, and optimising where necessary, it is possible to automate the procedure to increase throughput. Primerdesign has optimised on the KingFisher Flex magnetic particle processor (ThermoFisher Scientific) for 100 µL starting volumes.

4.1 – Automation on the KingFisher Flex

In addition to the components provided in the kit, the user needs to provide the following:

- Tips
- KingFisher deep-well plates (4 per extraction)
- KingFisher standard plates (2 per extraction)
- KingFisher comb (1 per extraction)
- Optional: Carrier molecule.

The optimised protocol for the KingFisher Flex has a total protocol time of 22 minutes. The protocols are shared with the manual extraction process, pending further individual laboratory optimisations.

When testing a protocol, it is important to observe for the following:

1. Tips that are potentially blocked.
2. Effective resuspension of the pellet after addition of each wash buffer.

5.0 – Troubleshooting

If issues are being observed with the exsig™ Mag kit, please refer to Section 5.1 for common troubleshooting solutions and Section 5.2 for frequently asked questions (FAQs). Alternatively, please contact our technical support team; techsupport@primerdsign.co.uk.

5.1 – Common troubleshooting solutions

Problem	Possible cause	Possible solution
PCR inhibition	Incomplete buffer removal	Ensure all buffer is completely removed before adding the next buffer in the procedure.
Low yield	Incomplete lysis	Contact our technical support team for assistance.
	RNA degradation before stabilised as cDNA	Store RNA at -80 °C. Use RNase free plastics.
	Sample is degraded	Store input sample at -80 °C prior to use.
	Inefficient binding	Ensure that the lysate, exsig™ Mag binding buffer, and exsig™ Mag beads are mixed thoroughly.
Particles present in eluate	Aspirating too fast	Reduce the speed at which supernatants are removed.
	Loose pellet	Increase magnetic separation or centrifugation time to allow formation of a tighter pellet.
	Disrupting pellet during aspiration	Position tip further away from pellet whilst removing supernatants.

Table 6. Common troubleshooting solutions for the exsig™ Mag kit.

5.2 – Frequently asked questions (FAQs)

Question	Possible solution
How do I safely inactivate biohazardous flow-through material?	Always dispose of potentially biohazardous solutions according to your institution's waste-disposal guidelines. Although the lysis and binding buffers in exsig™ Mag kits contain chaotropic agents that can inactivate some biohazardous material, local regulations dictate the proper way to dispose of biohazards. DO NOT add bleach or acidic solutions directly to the sample-preparation waste. The guanidine hydrochloride present in the sample- preparation waste can form highly reactive compounds when combined with bleach. Please access our safety data sheet (SDS) online for detailed information on the reagents for each respective kit.
Can I use both a water bath and an incubator for any heat steps?	Yes, both pieces of equipment are suitable. However, it should be noted that heat conduction occurs more efficiently in a water bath compared to an incubator. Incubation times may therefore have to be adjusted depending on the equipment used. Please contact our Technical Support Team for further advice.
What is the recommended method for assessing the quantity and quality of the purified viral RNA?	The recommended method for assessing the purified nucleic acid is through real-time quantitative PCR (RT-qPCR).
Once the nucleic acid is eluted, can the beads be reused?	Do not reuse the exsig™ Mag beads. There is risk of RNA carryover from one sample to the next. Use fresh exsig™ Mag beads for each sample.

Table 7. Frequently asked questions for the exsig™ Mag kit

6.0 – Safety Information

To access the SDS documents for the components in this kit, please contact our tech support team at techsupport@primerdesign.co.uk. Work with pathogens should be carried out according to the regulation of the country within which the kit is being used.

- Wear appropriate skin and eye protection throughout the preparation procedure.

- exsig™ Mag lysis buffer, exsig™ Mag binding buffer, and exsig™ Mag wash buffer 2 contain high concentrations of detergent and salt.
- exsig™ Mag binding buffer and exsig™ Mag wash buffer 2 contain up to 50% n-propanol, therefore keep away from naked flames.
- Ensure kit components are stored appropriately according to local safety guidance.
- In case of accidental contact, thoroughly rinse or flush the affected areas with water.
- Spillages can be removed using standard laboratory cleaning procedures.
- SDSs are available for all kit components on request.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
exsig™ Mag lysis buffer	 Warning	H302/H315/H319/H400	P101/P102/P103/P273/ P280/P305+P351+P338 / P301+P312/P332+P313 /P501/P301+P312
exsig™ Mag binding buffer	 Danger	H226/H302/H315/H318/H3 36/H400	P101/P102/P103/P210/ P241/P303+P361+P353 / P305+P351+P338/P310 /P501
exsig™ Mag particle suspension	-	-	-
exsig™ Mag wash buffer 1	 Danger	H226/H332/H315/H318/H3 36	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310 / P405/P501
exsig™ Mag wash buffer 2	 Danger	H315/H318/H226/H336	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310 /P405/ P501
exsig™ Mag wash buffer 3	-	-	-
exsig™ Mag elution buffer	-	-	-

Table 8. Safety information for exsig™ Mag kit components.

7.0 – Further Support

If you require any further assistance with this kit, please contact our technical support team at techsupport@primerdesign.co.uk and we will be happy to help.