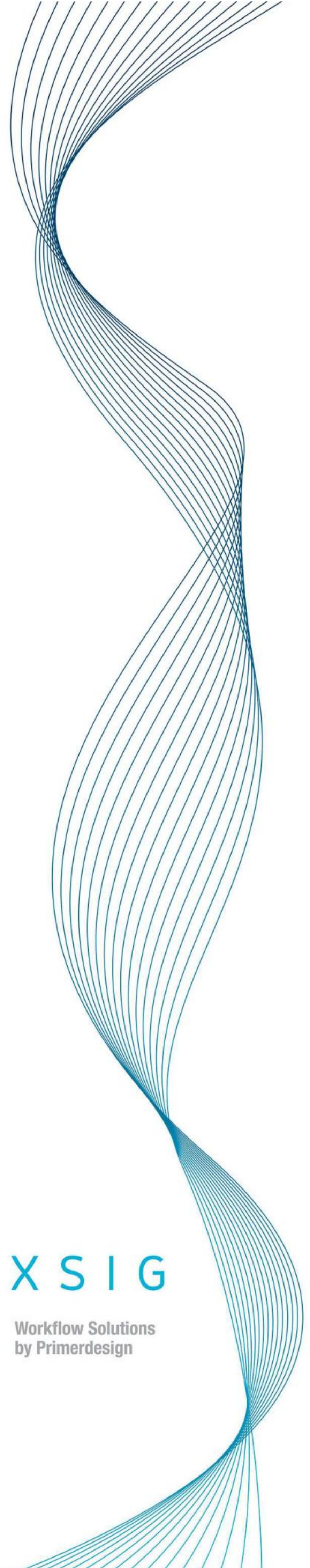
EXSIG



Primer Design Ltd

exsig®
Mag 96

96 extractions

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

R30096

Part of the NOVACYT GROUP

EXSIG

Workflow Solutions by Primerdesign

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1 – Introduction

exsig® Mag 96 from Primer Design Ltd uses magnetic separation for the purification of nucleic acid from various matrices such as clinical samples (e.g. nasopharyngeal swab, stool, blood, serum, plasma and sputum), veterinary, water, food and environmental material. 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 nucleic acid 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.

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

Component	Colour	Volume	Storage
exsig® Mag Lysis buffer	Blue	1 x 15 ml	Room temperature
exsig® Mag Binding buffer	Green	1 x 30 ml	Room temperature
exsig® Mag Particle suspension	White	1 x 2 ml	Room temperature; 4°C after opening
exsig® Mag Wash Buffer 1	Red	2 x 30 ml	Room temperature
exsig® Mag Wash Buffer 2	Red	2 x 30 ml	Room temperature
exsig® Mag Wash Buffer 3	Yellow	2 x 30 ml	Room temperature; 4°C after opening
exsig® Mag Elution buffer	Black	1 x 15 ml	Room temperature; 4°C after opening

3 – Experimental Procedure

3.1 – General Information before starting

When performing the exsig® Mag DNA/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:

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

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.

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 60°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.

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

STEP	Lysis*				Binding	Wash	Elution
Component	Optional: Protease solution (20µl)	Optional: Carrier molecule	Sample (100µl)	exsig® Mag Lysis buffer (100µl)	exsig® Mag binding buffer (160µl) + exsig® Mag Particle suspension (20µl)	exsig® Mag wash buffers 1. (400µl) 2. (400µl) 3. (400µl)	exsig® Mag elution buffer (100µl)
Condition	3 minutes, 55°C				5 minutes, room temperature + 2 minutes contact with magnet	1 minute room temperature + 2 minutes contact with magnet	5 minutes 60°C + 3 minutes contact with magnet

*Extended lysis duration (10 min) should be considered with stool samples

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
    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 3.3).
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 – 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 the exsig® Mag chemistry on the KingFisher Flex magnetic particle processor (ThermoFisher Scientific) and the CO-PREP Extraction System 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.
- Optional: Protease solution

The optimised protocol for the KingFisher Flex takes a total of 22 minutes. The protocol is consistent with the manual extraction process, pending further individual laboratory optimisations. Contact techsupport@primerdesign.co.uk to receive the protocol.

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

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

4.2 – Automation on the CO-PREP Extraction System

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

- CO-PREP ES Tips
- CO-PREP ES deep-well plates (1 plate for 16 extractions, machine can accommodate 3 plates for a total of 48 extractions)
- Optional: Carrier molecule
- Optional: Protease solution

The optimised protocol for the CO-PREP ES has a total protocol time of 35 minutes. Add the exsig® Mag components and sample(s) to the CO-PREP ES deep-well plate as outlined in Table 4 and Table 5. The optimised programme settings of the CO-PREP ES for use with exsig® Mag are described in Table 6. Contact techsupport@primerdesign.co.uk to receive the protocol that can be uploaded to the CO-PREP ES. Individual laboratory optimisation may be required depending on the sample type.

Table 4. CO-PREP ES Plate format

	Samples 1-8						Samples 9-16					
	1	2	3	4	5	6	7	8	9	10	11	12
A	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
B	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
C	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
D	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
E	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
F	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
G	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute
H	lysis	WB1	WB2	WB3		elute	lysis	WB1	WB2	WB3		elute

Table 5. Summary of exsig® Mag components for use with CO-PREP ES

Component	Column number*	Volume
exsig® Mag lysis buffer	1	100µl
exsig® Mag magnetic particle premix		180µl (160µl exsig® Mag binding buffer + 20µl exsig® Mag particle suspension)
Protease solution (optional)		20µl
Sample		100µl
exsig® Mag wash buffer 1	2	400µl
exsig® Mag wash buffer 2	3	400µl
exsig® Mag wash buffer 3	4	400µl
exsig® Mag elution buffer	6	100µl

*Columns 7-12 follow same format as above table to complete total of 16 extractions.

Table 6. Programme settings for CO-PREP ES with exsig® Mag reagents.

Temp1	Temp2
OFF	OFF

Well	Name	Volume	Action	Mixing	Collect
1	LB	400	For. U/D	Low	Low
2	WB1	400	For. U/D	Low	Low
3	WB2	400	For. U/D	Low	Low
4	WB3	400	For. U/D	Low	Low
5	N/A	0	For.	Low	Low
6	EB	100	For.	Low	Low

Step	Well	Temp	Mix time	Mix speed	Collect time	Vapor time	Pause
1	1	Off	5	3000	2*	0	Off
2	2		1	3000	2*	0	Off
3	3		1	3000	2*	0	Off
4	4		1	3000	2*	5	Off
5	6	100	3	1500	3*	0	Off

*For more challenging sample types e.g. plasma, increasing bead collection time to 5 min should be considered to improve transfer of the beads between wells.

5 – 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

Table 7. Common troubleshooting solutions for the exsig® Mag kit.

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.

5.2 – Frequently asked questions (FAQs)

Table 8. Frequently asked questions for the exsig® Mag kit

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 refer to the safety data sheet (SDS) 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 DNA/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 DNA/RNA carryover from one sample to the next. Use fresh exsig® Mag beads for each sample.

6 – 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.

Table 9. Safety Information for Exsig® Mag kit components

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/H336	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	-	-	-

7 – 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.