EXSIG

Primer Design Ltd

exsig®Mag RapidBead Pro

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

R01367 - exsig® Mag RapidBead Pro Nucleic Acid Extraction Kit (50)

R01368 - exsig® Mag RapidBead Pro Nucleic Acid Extraction Kit (150)

Part of the NOVACYT GROUP

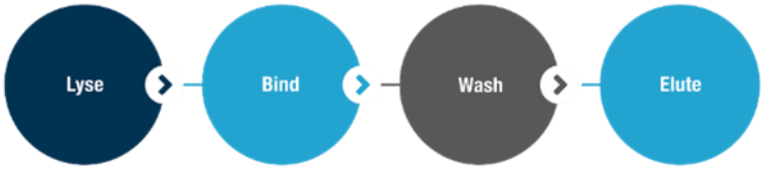
EXSIG

Workflow Solutions by Primerdesign

Contents

1. Introduction

exsig® Mag RapidBead Pro from Primer Design Ltd uses magnetic separation for the purification of nucleic acid from various clinical sample matrices, e.g., anterior nasal swab, stool, serum, plasma and saliva. The exsig® Mag RapidBead Pro surface activated magnetic particles are specifically designed to capture nucleic acids with high affinity, and combined with a single wash step, allow efficient removal of impurities present in the sample matrix. After washing, the nucleic acid is eluted and is ready for use in downstream applications.



This kit is intended for general laboratory and 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. The kit is shipped at ambient temperature. The exsig® Mag RapidBead Pro Magnetic Beads are stable at ambient for short term storage but should be removed upon receipt and stored at 2-8°C. Do not freeze.

Table 1: exsig® Mag RapidBead Pro kit (50 reactions / 150 reactions) components and storage conditions.

Component	Volume (50)	Volume (150)	Storage
Lysis Buffer	1 x 5.5 mL	1 x 16.5 mL	15-25°C
Binding Buffer	1 x 22 mL	1 x 66 mL	15-25°C
Wash Buffer	1 x 33 mL	1 x 99 mL	15-25°C
Elution Buffer	1 x 5.5 mL	1 x 16.5 mL	15-25°C
Magnetic Beads	1 x 1.1 mL	1 x 3.3 mL	2-8°C

3. Experimental Procedure

3.1. General information before starting

When performing the exsig® Mag RapidBead Pro DNA/RNA purification protocol using the manual protocol, a heated shaker and a magnetic rack suitable for 1.5mL tubes are required.

exsig® Mag RapidBead Pro is fully compatible with both automated and manual workflows. The automated method has been optimised for use on the KingFisher Flex. Instructions for obtaining the optimised protocol for the KingFisher Flex (Thermo Fisher) can be found in section 4.

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

It is important to ensure that the Magnetic Beads are completely resuspended before adding to the Binding Buffer. Use of non-homogenous Magnetic Beads will affect the efficiency of the purification chemistry, potentially resulting in lower yields.

For optimal performance, the considerations in Table 2 should be applied to the experimental process when performing the relevant steps.

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

Protocol process	Consideration
Bring magnetic rack into contact with tubes	The Magnetic Beads will form a pellet on the side of the tube, to allow for easy removal of the supernatant. The times stated for magnetic bead pelleting are minimum recommended incubation times. The strength of the magnetic rack will influence the speed of Magnetic 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 Magnetic 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 Magnetic Beads do not settle. This movement will increase the efficiency of the binding and washing steps.

3.2. Required Materials

3.2.1. Manual Procedure

a. Magnetic rack
b. Reaction tubes that are RNase-free
c. Pipettes and tips with a range of suitable volumes
d. Shaking heat block with a range up to 70°C
e. Vortex
f. Mini centrifuge

3.2.2. Automated Procedure

a. KingFisher Flex
b. Pipettes and tips with a range of suitable volumes
c. KingFisher deep-well plates (4 per extraction)
d. KingFisher comb (1 per extraction)

3.3. Initial Preparations

3.3.1. Reagent Preparation

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 Magnetic Beads: the Magnetic Beads and Binding Buffer can be added to the reaction(s) as a premix. Follow the steps below to prepare the premix:

    i. Thoroughly mix the Magnetic Beads to fully resuspend the particles.
    ii. In a clean tube, add 20µL Magnetic Beads to 400µL Binding Buffer for each sample to be extracted.
    iii. If preparing premix for multiple reactions, multiply the volumes accordingly, allowing sufficient overage.

3.3.2. Sample Preparation

100µL of sample is required for the procedure. Liquid saliva, plasma and serum samples require no further sample processing. For stool and anterior nasal swabs, see the recommendations in section 8.

3.4. Overview of the manual exsig® Mag RapidBead Pro purification protocol

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

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

STEP	Lysis		Binding	Wash	Elution
Component	Sample in liquid (100µL)	Lysis Buffer (100µL)	Binding Buffer (400µL) + Magnetic Beads (20µL)	Wash Buffer (600µL)	Elution Buffer (100µL)
Condition 1	55°C with shaking **3 minutes**		Ambient with shaking **5 minutes**	Ambient with shaking **2 minutes**	70° with shaking **5 minutes**
Condition 2	N/A		Contact with magnet **2 minutes**	Contact with magnet **2 minutes**	Contact with magnet **2 minutes**
Total Step Time	3 minutes*		7 minutes	4 minutes	7 minutes

*Extended lysis duration (10 minutes) 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. 100µL of the liquid starting sample.
    b. 100µL Lysis Buffer.
    c. Pulse vortex and quickly spin down the sample.
    d. Optional step: addition of 20µL Proteinase K (not included).
    e. Optional step: addition of Internal Extraction Control.
2. Incubate at 55°C for 3 minutes with constant shaking.
3. Allow the sample(s) to cool to room temperature.
4. Add 20µL Magnetic Beads and 400µL Binding Buffer (or add 420µL of premix – see section 3.3).
5. Mix thoroughly by pulse vortexing, gently spin down 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 600µL Wash Buffer.
10. Resuspend the Magnetic Beads in the Wash Buffer.
11. Incubate for 2 minutes at room temperature, with constant shaking.
12. Bring magnet into contact with the tube(s) for 2 minutes.
13. Remove the supernatant and discard.
14. Separate the magnet from the sample tube(s).
15. Add 100µL Elution Buffer.
16. Resuspend the Magnetic Beads in the Elution Buffer by pipetting up and down.
17. Incubate for 5 minutes at 70°C with constant shaking.
18. Bring magnet into contact with the tube(s) for 2 minutes.
19. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any Magnetic Beads.

4. Automating the nucleic acid purification protocol

Primer Design has optimised the exsig® Mag RapidBead Pro chemistry for use on the KingFisher Flex magnetic particle processor (ThermoFisher Scientific).

4.1. Automation on the KingFisher Flex

The optimised protocol for the KingFisher Flex takes a total of 17 minutes. The protocol is consistent with the manual extraction process. Contact techsupport@primerdesign.co.uk to receive the protocol.

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

1. Tips that are potentially blocked.
2. Effective resuspension of the pellet after addition of Wash Buffer.

5. Troubleshooting

If issues are being observed with the exsig® Mag RapidBead Pro 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@primerdesign.co.uk

5.1. Common troubleshooting solutions

Table 4: Common troubleshooting solutions for the exsig® Mag RapidBead Pro 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, Binding Buffer, and Magnetic Beads are mixed thoroughly.
Particles present in eluate	Aspirating too fast.	Reduce the speed at which supernatants are removed.
	Loose pellet.	Increase magnetic separation 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 5: Frequently asked questions regarding the exsig® Mag RapidBead Pro kit.

Question	Primer Design recommendation
How do I safely inactivate biohazardous waste material?	Always dispose of potentially biohazardous solutions according to your institution's waste-disposal guidelines. Although the Lysis and Binding Buffers in exsig® Mag RapidBead Pro 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 component.
How should the quantity and quality of the purified DNA/RNA be assessed?	The recommended methods for assessing the purified nucleic acid are by quantification on a suitable spectrophotometer or through real-time quantitative PCR (RT-qPCR).
Once the nucleic acid is eluted, can the beads be reused?	Do not reuse the Magnetic Beads. There is risk of DNA/RNA carryover from one sample to the next. Use fresh Magnetic Beads for each sample.

6. Safety Information

The SDS document for this kit can be found on our website (Safety Data Sheets), alternatively, 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.
- The kit Lysis Buffer, Binding Buffer and Wash Buffer contain high concentrations of detergent and salt.
- The kit Wash Buffer contains ethanol, 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.

Table 6: Safety Information for exsig® Mag RapidBead Pro kit components.

Kit component	GHS symbol and signal word	Hazard phrases	Precaution phrases
Lysis Buffer	 Health Hazard Serious Health Hazard **Warning/Danger**	H302/H315/ H319/H400	P101/P102/P103/P273/P280/ P305+P351+P338/P301+P312/ P332+P313 /P501/P301+P312
Binding Buffer	 Health Hazard/Flammable **Danger**	H226/H302/H315/ H318/H3 36/H400	P101/P102/P103/P210/P241/ P303+P361+P353/P305+P351+ P338/P310 /P501
Magnetic Beads	Not applicable	None	None
Wash Buffer	 Health Hazard/ Serious Health Hazard/Flammable **Danger**	H226/H332/ H315/H318/H336	P101/P102/P103/P210/P303+ P361+P353/P305+P351+P338/ P310 /P405/P501
Elution Buffer	Not applicable	None	None

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.

8. Method for non-liquid samples

Table 7: Alternative sample types.

Sample Type	Preparation Instructions
Anterior Nasal Swab	1. Prepare a 1.5mL tube with 1000µL PBS. 2. Insert cotton swab ~2cm into the nostril and rotate for 15 seconds, repeat for the second nostril. 3. Insert the swab into the PBS containing tube and swirl for 15 seconds. 4. Pulse vortex to ensure complete mixing then briefly spin.
Stool	1. Prepare a 1.5mL tube with 1000µL PBS. 2. Insert swab into the stool sample and swirl 5 times to ensure the head of the swab is suitably covered in stool material. 3. Submerge swab into the 1.5mL tube containing PBS and swirl for a further 15 seconds. 4. Pulse vortex to ensure complete mixing then briefly spin.