MAGFLO PCR Instruction Manual

INTEGRA

MAGFLO™ PCR

Magnetic beads for PCR purification

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INTEGRA

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1. How to use MAGFLO PCR for PCR purification

1.1 Intended use

MAGFLO PCR magnetic beads are intended for **research use only (RUO)**, for molecular biology research. They are not intended or validated for use in the diagnosis of disease or other medical conditions. They are designed to be used manually or with liquid handling automation for molecular biology applications.

1.2 Symbols used in the document

The instruction manual specifically advises of residual risks with the following symbol:

Warning: This safety symbol warns against hazards that could result in injury. It also indicates hazards for machinery, materials and the environment. It is essential that you follow the corresponding precautions.

Note: This symbol identifies important notes regarding the correct operation of the reagent and labor-saving features.

Table 1: Symbols found on the packaging of MAGFLO PCR magnetic beads.

	QR code for instruction manual and SDS access
	Storage temperature limit
	Expiration date
	Lot number
	Manufacturer information

1.3 Safety notes

Please consult the material safety data sheet (SDS) for all safety and disposal information. This can be accessed via the QR code on the packaging.

Note: According to the SDS, MAGFLO PCR magnetic beads are not classified as a hazardous substance, therefore there are no precautionary statements for prevention or response related to this product.

Warning: Always follow your facility's procedures and universal precautions – by using disposable gloves, safety glasses, a lab coat, etc. – when working with chemicals.

1.4 What is inside MAGFLO PCR reagent

MAGFLO PCR magnetic beads consist of superparamagnetic particles in a binding buffer.

Note: Read the instructions carefully before using the kit.

Note: Please consult your local waste regulations for information about safe disposal.

2. Application of MAGFLO PCR magnetic beads

MAGFLO PCR magnetic beads offer an effective solution for the purification of amplicons during PCR clean-up. Purified nucleic acids are eluted using a low salt elution buffer or molecular biology grade water, and can be used directly in downstream applications.

The protocol can easily be carried out using an INTEGRA MAG module magnetic separation device, eliminating the need to manually move the plate onto and off of the magnet. MAG modules use vertically moving magnetic arrays, so the plate stays in one place during magnetization steps. Magnetic bead purification workflows can also be automated on the ASSIST PLUS pipetting robot, or using a VIAFLO 96 or VIAFLO 384 electronic pipette for streamlined liquid handling.

Performance features

- Designed for DNA amplicons.
- High recovery of amplicons larger than 100 bp during PCR clean-up.
- Efficiently removes excess primers, primer dimers, unincorporated nucleotides, salts and enzymes.
- No centrifugation or filtration step needed.

Sample input requirements

- Samples should contain a double-stranded DNA amplicon.

Nucleic acid fragments are ready to use for the following downstream applications

- PCR/qPCR/ddPCR
- Mutation detection and genotyping
- Sanger sequencing protocols
- Fragment analysis
- Microarrays
- Enzymatic reactions
- Cloning
- Transfection experiments
- Ligation

2.1 Introduction to PCR purification

MAGFLO PCR magnetic beads offer reliable purification of PCR amplicons at a specific bead-to-sample ratio of 1.8. The magnetic beads selectively bind DNA fragments larger than 100 bp. The PCR purification process involves three simple steps – bind, wash and elute – during which magnetic beads bind the DNA fragments of ≥ 100 bp, while eliminating excess primers, primer dimers, unincorporated nucleotides, salts and enzymes. Fragments of interest are then recovered in an elution buffer (**Figure 1**).

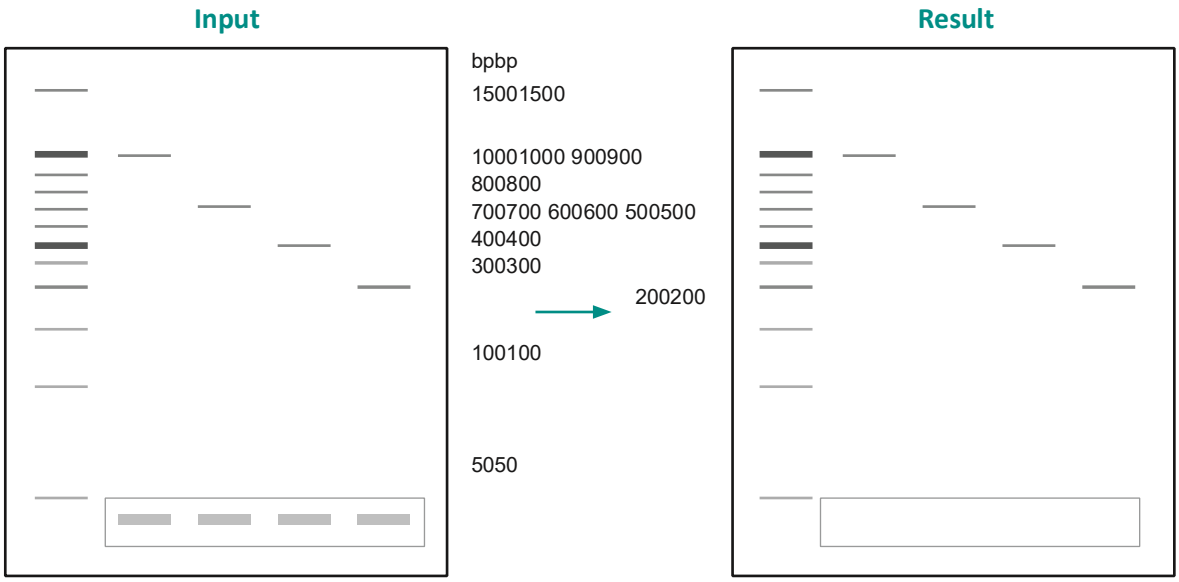

Figure 1: Schematic overview of the PCR purification process illustrated with a DNA ladder. During this process, small fragments – representing primers and/or primer dimers (below 100 bp) – are removed, and different sized amplicons are isolated.

3. Before you start

3.1 Reagent shipping, storage and handling

MAGFLO PCR magnetic beads are stable during shipment at ambient temperatures, but cannot be frozen. The recommended storage temperature is 2-8 °C. Bring the product to room temperature (RT) before use.

Note: MAGFLO PCR magnetic beads are no longer suitable for use if they have been frozen.

Note: Do not use the product after the expiration date stated on the label.

3.2 Quality control

MAGFLO PCR magnetic beads are produced according to predetermined and validated protocols in the quality management system. Additionally, a quality check is performed after the production of each lot. This is documented in the certificate of conformance to ensure consistent product quality.

Note: The certificate of conformance is available upon request. Please reach out to your regional INTEGRA representative.

3.3 Additional materials and reagents

Materials and reagents to be supplied by the user.

Equipment

- Magnetic separation device (e.g. INTEGRA MAG/HEATMAG module)
- Pipettes (manual or electronic)
- A liquid handling system (e.g. ASSIST PLUS, VIAFLO 96 or VIAFLO 384)

Note: We recommend using the ASSIST PLUS with an integrated MAG/HEATMAG module for full walk-away magnetic bead purification. In this set-up, the software fully integrates and automates the magnetic separation step by controlling the up and down movement of the magnetic array.

Note: We recommend combining the VIAFLO 96 or VIAFLO 384 with a MAG/HEATMAG module for semi-automated magnetic separation without labware transfer. In this set-up, the magnetic separation step is fully automated by moving the magnetic array up and down.

Note: We recommend using VIAFLO multichannel electronic pipettes or EVOLVE manual pipettes with a standalone MAG/HEATMAG module for manual workflows. In this set-up, the magnetic separation step is fully automated by moving the magnetic array up and down.

Consumables

- Source labware of your choice – such as a 96 or 384 well microplate, 8 well PCR strips, or microcentrifuge tubes
- Destination labware of your choice – such as a 96 or 384 well microplate, 8 well PCR strips, or microcentrifuge tubes
- Pipette tips
- Reagent reservoirs

Note: We recommend the Bio-Rad Hard-Shell ® 96-Well PCR Plate (HSP9601) for optimal performance. INTEGRA deep well plates (6535) are recommended for reliable pipetting of reagents on INTEGRA liquid handling systems, or if the reaction volume exceeds the volume of the PCR plate. Additionally, we recommend low retention, sterile, filter GRIPTIPS ® to handle reagents used in the protocol.

Reagents

- 70 % ethanol (freshly prepared from non-denatured alcohol) for the washing steps
- Molecular biology grade water (DNase-free) or elution buffer (10 mM Tris-HCl, pH 8.0) for the elution step

Note: It is important to prepare a fresh solution of 70 % ethanol every time. Storing the solution before use may impact washing step efficacy and negatively affect results.

3.4 Prepare reagents

- Make sure you prepare the 70 % ethanol fresh before use.
- Bring MAGFLO PCR magnetic beads to RT and vortex them thoroughly to fully resuspend the magnetic particles prior to use.

4. Protocol

4.1 PCR purification

The protocol provided is valid for 96 or 384 well plate formats, microcentrifuge tubes, and 2.2 ml deep well plates.

1. Use a 1.8x bead-to-sample ratio for PCR clean-up.
2. Bring MAGFLO PCR magnetic beads to RT and vortex them thoroughly to fully resuspend the magnetic particles prior to use.
3. Measure the reaction volume of the sample(s), and determine if it is necessary to transfer the sample(s) to a suitable processing plate or tube.
4. Add 1.8x the reaction volume of MAGFLO PCR magnetic beads to each well. An example of the respective bead volume can be found in **Table 2** below.

Input sample volume × ratio = volume of magnetic beads
Example: 50 µl × 1.8 = 90 µl of magnetic beads

5. Pipette up and down 5-20 times or vortex for 30 seconds until the solution appears homogeneous.
6. Incubate at RT for 5 minutes.
7. Engage the magnet and separate the magnetic beads. Incubate at RT until the solution is completely cleared of magnetic beads and the bead pellet is formed.
8. Aspirate and discard the cleared supernatant. Do not disturb the magnetic bead pellet.
9. Add the appropriate volume of fresh 70 % ethanol to each well as follows:
   30 µl for a 384 well plate200 µl for a 96 well plate; 500-1000 µl for a microcentrifuge tube or deep well plate
10. Incubate at RT for 1 minute without resuspending the pellet.
11. Aspirate and discard the cleared supernatant. Do not disturb the magnetic bead pellet.
12. Repeat steps 9-11 to complete a second 70 % ethanol wash step.
13. Keeping the magnet engaged, remove any residual liquid, and air dry the magnetic beads for 3-15 minutes. Ensure any residual liquid is removed.
14. Disengage the magnet and add the appropriate volume – between 10 and 100 µl – of molecular biology grade water or elution buffer to each well (e.g. 10 µl sample and 10 µl elution volume represents a 1:1 dilution).
15. Pipette up and down 20-30 times or vortex for 30 seconds until the solution appears homogeneous.
16. Incubate at RT for 5 minutes.
17. Engage the magnet and separate the magnetic beads. Incubate at RT until the solution is completely cleared of magnetic beads.
18. Transfer the cleared supernatant containing purified DNA to a new plate or tube, and store the eluates at 2-8 °C for short-term storage, or -20 °C for long-term storage.

Table 2: Volumes for a 1.8x MAGFLO PCR-to-sample ratio.

POSSIBLE LABWARE	PCR REACTION VOLUME (µl)	MAGNETIC BEADS VOLUME (µl)
96 well PCR plate	10	18
	20	36
	50	90
384 well PCR plate	5	9
	7	12.6
	10	18
Microcentrifuge tube	50	90
	100	180
	150	270

5. Troubleshooting guide

Please use this guide to troubleshoot some known problems that may arise. Contact your regional INTEGRA sales representative or field application specialist for further assistance.

Table 3: Troubleshooting guide.

PROBLEM	CAUSE	SOLUTION
Low yield	Inefficient PCR reaction	Increase the number of amplification cycles for PCR and/or further optimize the PCR reaction.
	Smaller product size (bp)	Small DNA fragments normally give lower yield.
	Ethanol residue interference	During the drying step, remove any liquid from the bottom of the well. Make sure to use fresh 70 % ethanol.
	Magnetic bead loss during the procedure	Increase magnetization time. Aspirate slowly. Make sure the plate or tube fits well on the magnet.
	DNA remains bound to magnetic beads	Prevent over drying the particles and/or increase the elution volume.
	Incomplete resuspension of the magnetic beads during elution	Vortex or pipette up and down to fully resuspend the particles. Increase the number of mixing cycles. To increase yield, you can also heat the elution buffer up to 65 °C before use. Reduce the drying time to prevent over drying the beads.
Primer carryover	Insufficient washing of the magnetic beads	Wash the magnetic beads one more time with 70 % ethanol. Make sure to use freshly prepared ethanol.
Non-specific amplification products were not removed	The size of the non-specific amplification products is larger than 100 bp	Non-specific amplification products larger than 100 bp are not efficiently removed from PCR products in the standard protocol (1.8x ratio). Optimization of the bead-to-sample ratio might be required.
Problems in downstream applications	Salt carryover	70 % ethanol must be stored at RT.
	Ethanol carryover	Ensure all traces of ethanol are removed after each ethanol wash and the magnetic beads are completely dried before elution. Make sure to use fresh 70 % ethanol.

6. User insights on INTEGRA's benchtop pipetting solutions

6.1 High throughput solutions with MAGFLO PCR magnetic beads

If you are interested in processing a complete plate of samples, please refer to the workflow set-up that combines a VIAFLO 96 or VIAFLO 384 handheld electronic pipette with a magnetic separation module (MAG/HEATMAG). A detailed script with optimized pipetting parameters is available for download in the application note on our website.

Note: If you want to modify the program, please refer to handout to change the sample input and the elution volume for PCR purification on VIAFLO 96 and VIAFLO 384, available upon request from your regional INTEGRA sales representative.

Note: The scripts for PCR clean-up using a VIAFLO 96 or VIAFLO 384 handheld electronic pipette, equipped with the 125 µl or 300 µl 96 channel pipetting head, are provided in manual and automatic mode.

7. Ordering information

Contact your regional INTEGRA sales representative to place an order.

Table 4: Article numbers available for MAGFLO PCR magnetic beads.

ARTICLE NUMBER	DESCRIPTION	NUMBER OF REACTIONS FOR PCR CLEAN-UPS*
7010	MAGFLO PCR magnetic beads, 1 ml	~ 55
7012	MAGFLO PCR magnetic beads, 50 ml	~ 2'777
7014	MAGFLO PCR magnetic beads, 500 ml	~ 27'777

*Number of reactions is based on a 10 µl PCR reaction volume. For PCR product purification with a bead-to-sample ratio of 1.8x, the volume of magnetic beads to be used per reaction is 10 x 1.8 = 18 µl

8. Imprint

INTEGRA Biosciences AG aims to provide reliable and accurate data and documentation. If you find a discrepancy, we would be grateful for your assistance and ask you for email us at info@integra-biosciences.com.

This operating instruction manual has part number 137963, the version is V00.

Manufacturer and customer service

Manufactured for INTEGRA Biosciences AG by CleanNA BV (Coenecoop 75, 2741 PH Waddinxveen, The Netherlands). Your local INTEGRA Biosciences representative, further information, and this instruction manual in other languages can be found at www.integra-biosciences.com, or are available on request from info@integra-biosciences.com.

INTEGRA Biosciences AG
Tardisstrasse 201
CH-7205 Zizers, Switzerland
T +41 81 286 95 30
info-ch@integra-biosciences.com

INTEGRA Biosciences Ltd
2 Rivermead Business Park
Thatcham, Berks, RG19 4EP, United Kingdom
T +44 1635 797 00
info-uk@integra-biosciences.com