INTEGRA

MAGFLO™ NGS

Magnetic beads for NGS size selection

Table of content

1 How to use MAGFLO™ NGS for NGS size selection 3
1.1 Intended use 3
1.2 Symbols used in the document 3
1.3 Safety notes 3
1.4 What is the MAGFLO™ NGS reagent 4
2 Application of MAGFLO™ NGS magnetic beads 4
2.1 Introduction to single- and double-sided size selection NGS 5
3 Before you start 6
3.1 Reagent shipping, storage and handling 6
3.2 Quality control 6
3.3 Additional materials and reagents 6
3.4 Reagent preparation 7
3.5 Precautions to avoid RNase contamination 7
4 Protocol 8
4.1 Single-sided size selection 8
4.2 Double-sided size selection 10
5 Troubleshooting guide 12
6 User insights on INTEGRA's benchtop pipetting solutions 13
6.1 Walk-away solutions for MAGFLO™ NGS magnetic beads 13
7 Ordering information 14
8 Disclaimer 14

1 How to use MAGFLO™ NGS for NGS size selection

1.1 Intended use

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

1.2 Symbols used in the document

The instruction manual specifically advises of residual risks using the following symbols:

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 use of the reagent and labor-saving features.

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

***Table 1**: Symbols found on the packaging of MAGFLO™ NGS magnetic beads.*

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™ NGS 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 and use disposable gloves, safety glasses, a lab coat, etc. when working with chemicals.

1.4 What is the MAGFLO™ NGS reagent

MAGFLO™ NGS 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™ NGS magnetic beads

MAGFLO™ NGS magnetic beads offer an effective solution for the isolation of nucleic acid fragments with consistent size distribution, which is crucial for the library preparation step in next generation sequencing (NGS) workflows. This includes both single- and double-sided size selection methods. The MAGFLO™ NGS is manufactured under RNase-free conditions, allowing purification of RNA and DNA. 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 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 both DNA and RNA purification.
- Using a precise bead-to-sample ratio allows effective targeting of specific sized nucleic acid fragments.
- Ideal for single- and double-sided size selection steps used in NGS library preparations.
- No centrifugation or filtration step needed.

Sample input requirements

- Samples should contain fragmented double-stranded DNA or RNA.
- For the best performance in double-sided size selection:
- Optimal sample volume should be ≥50 µl. Lower volumes could decrease accuracy when pipetting beads, increasing variability.
- The desired fragment size should be between 100 and 1000 bp.
- The left-side ratio needs to be greater than the right-side ratio.

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

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

2.1 Introduction to single- and double-sided size selection NGS

Library preparation kits typically include protocols specifying the ratios (volumes) of magnetic beads to be used to selectively bind and purify DNA fragments of the desired average base pair (bp) size. MAGFLO™ NGS magnetic beads feature a single magnetic bead-to-sample ratio in single size selection, and two specific ratios in double-sided size selection. These ratios can be found in **Table 3** and **Table 4,** respectively.

Double-sided size selection consists of a right-sided single size selection (performed first), followed by a left-sided size selection (performed second) (**Figure 1**).

Right-sided, single size selection (large fragment removal): The first addition of magnetic beads binds nucleic acid fragments larger than the targeted average size. Engaging the magnet then captures the beads, and the supernatant – containing DNA fragments of target size and smaller – is recovered by transferring it to another well (**Figure 1**).

Left-sided, single size selection (small fragment removal): The second addition of magnetic beads binds fragments of the targeted average size. Engaging the magnet then captures the beads, and the supernatant – containing the smaller DNA fragments – is removed. A simple washing procedure then removes the remaining impurities. Fragments of interest are easily eluted by disengaging the magnet, and are then recovered after another separation (**Figure 1**).

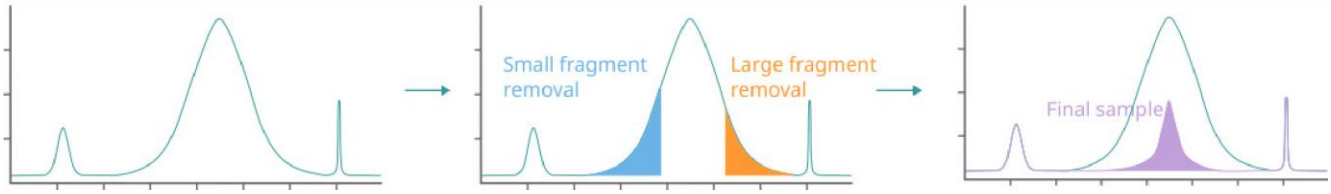

***Figure 1**: Schematic overview of size selection procedures.*

3 Before you start

3.1 Reagent shipping, storage and handling

MAGFLO™ NGS magnetic beads are stable during shipment at ambient temperatures. For maximum shelf life until the specified expiration date, storage at 2–8 °C is recommended. The beads can be stored stable at 15-25°C (room temperature) for up to one year, until the expiration date indicated on the product label. Bring the product to room temperature (RT) before use.

Note: Do not freeze MAGFLO™ NGS. After the MAGFLO™ NGS has been frozen, it is no longer suitable for use.

Note: Do not use the product after the expiration date stated on the label. Once stored at 15-25°C only the expiration date for 15-25°C is applicable.

3.2 Quality control

MAGFLO™ NGS 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 using low retention, sterile, filter GRIPTIPS® to handle the reagents used in the protocol.

Reagents

- 80 % 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 80 % ethanol every time. Storing the solution before use may impact the washing step efficacy and negatively affect results.

3.4 Reagent preparation

- Prepare 80 % ethanol fresh before use
- If the beads were refrigerated, bring the MAGFLO™ NGS magnetic beads to RT and vortex them thoroughly to fully resuspend the magnetic particles prior to use

3.5 Precautions to avoid RNase contamination

It is important to work RNase-free for RNA applications. The most common sources of RNases are hands, dust particles, and contaminated laboratory solutions, equipment, and glassware. Precautions should be taken to avoid the introduction of RNases while working with RNA.

Recommended precautions

- Always wear gloves when handling RNA samples. Change your gloves frequently to avoid contamination.
- Ensure that you use RNase-free filter tips when pipetting.
- Use disposable consumables that are guaranteed to be RNase-free.
- Use reagents that are guaranteed to be RNase-free. Creating aliquots from buffers lowers the risk of RNase contamination in buffers, reagents, etc.
- Avoid using reagents, consumables and equipment intended for common use or general lab processes.
- If possible, work in a separate room, fume hood or lab space.
- Clean all working surfaces with commercial RNase-inhibiting surfactants or 80 % ethanol before starting your work.

Note: All INTEGRA consumables – including tips and reservoirs – are certified to be RNase-free.

4 Protocol

4.1 Single-sided size selection

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

1. Choose the magnetic bead ratio (e.g. 1.8x ratio for PCR clean-up) for your size selection step according to **Table 2**. Select the desired fragment cutoff shown in Figure 2 that corresponds to the target fragment size listed in Table 3.
2. Bring MAGFLO™ NGS 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 the desired volume of MAGFLO™ NGS magnetic beads to each well. For PCR clean-up, add 1.8x the reaction volume of MAGFLO™ NGS magnetic beads to each well. An example of the respective bead volumes 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 to 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 80 % ethanol to each well as follows:
   30 µl for a 384 well plate; 125-180 µl for a 96 well plate; 500-1000 µl for a microcentrifuge tube.
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 80 % 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 3-5 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 or RNA to a new plate or tube and store the eluates at 2-8 °C for short-term storage, or DNA at -20 °C and RNA at -80 °C for long-term storage.

Possible labware	Ratio 0.6x		Ratio 0.9x		Ratio 1.8x	
	Reaction volume (µl)	Magnetic beads volume (µl)	Reaction volume (µl)	Magnetic beads volume (µl)	Reaction volume (µl)	Magnetic beads volume (µl)
96 well PCR plate	10	6	10	9	10	18
	20	12	20	18	20	36
	50	30	50	45	50	90

***Table 2**: Volumes for a specific MAGFLO™ NGS-to-sample ratio.*

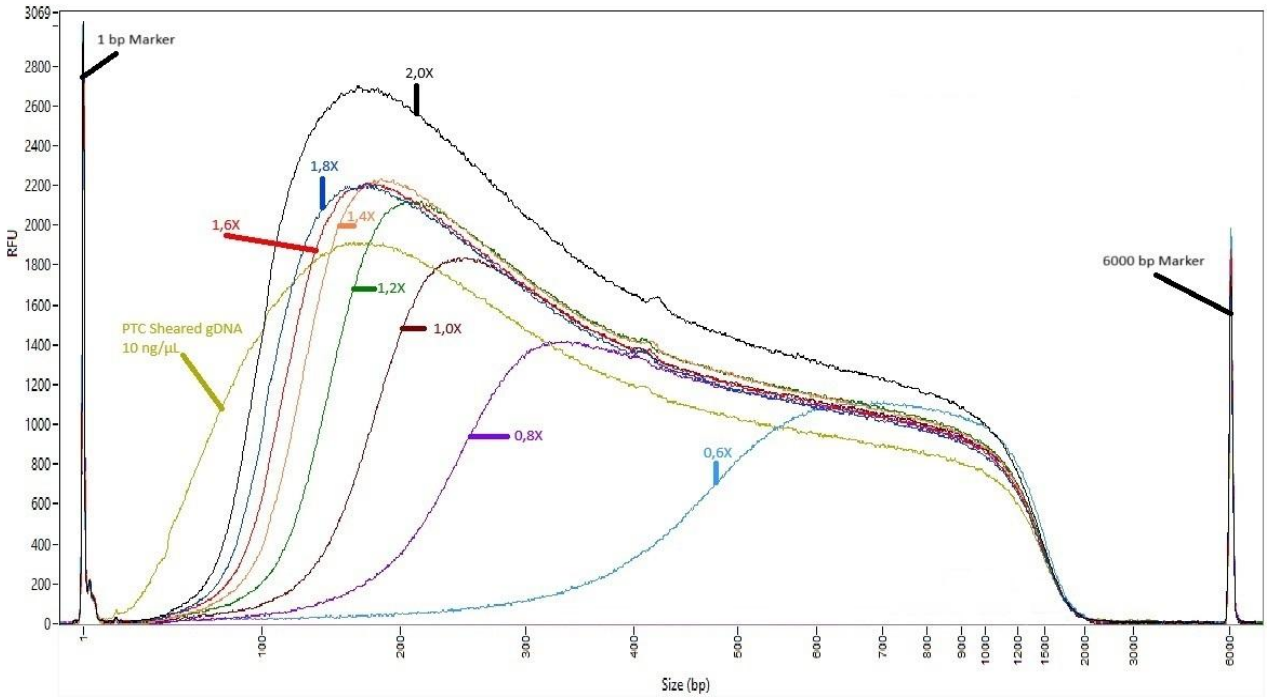

***Figure 2**: Electropherogram traces of sheared gDNA purified using MAGFLO™ NGS magnetic beads, showing the resulting cutoff based on the tested MAGFLO™ NGS bead-to-sample ratio (Table 3).*

Product	Ratio	Peak start (bp)
MAGFLO™ NGS	2.0x	~70 bp
MAGFLO™ NGS	1.8x	~80 bp
MAGFLO™ NGS	1.6x	~85 bp
MAGFLO™ NGS	1.4x	~95 bp
MAGFLO™ NGS	1.2x	~110 bp
MAGFLO™ NGS	1.0x	~140 bp
MAGFLO™ NGS	0.8x	~185 bp
MAGFLO™ NGS	0.6x	~350 bp

***Table 3**: Cut-off values corresponding to the electropherogram traces of sheared gDNA (Figure 2) purified using MAGFLO™ NGS magnetic beads, indicating the anticipated lower fragment limit.*

4.2 Double-sided size selection

The protocol provided is valid for 96 well plate formats and microcentrifuge tubes.

1. Choose a bead-to-sample ratio according to the desired nucleic acid fragment of interest (**Table 4**).
2. Bring MAGFLO™ NGS 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 the desired first volume of magnetic beads to each well (right-sided size selection).

Starting sample volume × ratio (right) = volume of magnetic beads
Example: 50 µl × 0.7 = 35 µl of magnetic beads

5. Pipette up and down 10-20 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 magnetic beads are completely cleared from the solution.
8. Transfer the cleared supernatant – containing small fragments and those of interest – to a new well.
9. Disengage the magnet and add the desired second volume of magnetic beads to each well (left-sided size selection).

Starting sample volume × ((ratio (left) - ratio (right)) = volume of magnetic beads
Example: 50 µl × (0.8-0.7) = 5 µl of magnetic beads

10. Pipette up and down 5-15 times or vortex for 30 seconds until the solution appears homogeneous.
11. Incubate at RT for 5 minutes.
12. Engage the magnet and separate the magnetic beads. Incubate at RT until the magnetic beads are completely cleared from the solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the magnetic bead pellet.
14. Add the respective volume of fresh 80 % ethanol to each well as follows:
15. 125-180 µl for a 96 well plate or 500-1000 µl for a microcentrifuge tube.
16. Incubate at RT for 1 minute without resuspending.
17. Aspirate and discard the cleared supernatant. Do not disturb the magnetic beads.
18. Repeat steps 14-16 to complete a second 80 % ethanol wash step.
19. Keeping the magnet engaged, remove any residual liquid, and air dry the magnetic beads for 3-15 minutes. Ensure any residual liquid is removed.
20. 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).
21. Pipette up and down 20 times or vortex for 30 seconds until the solution appears homogeneous.
22. Incubate at RT for 3-5 minutes.
23. Engage the magnet and separate the magnetic beads. Incubate at RT until the solution is completely cleared from the magnetic beads.
24. Transfer the cleared supernatant containing purified DNA or RNA to a new plate or tube and store the eluates at 2-8 °C for short-term storage, or DNA at -20 °C and RNA at -80 °C for long-term storage.

Bp target region	Ratio used (right/left)
180 – 1300	0.50/0.90
200 – 700	0.56/0.85
220 – 530	0.70/0.80
235 – 660	0.61/0.80
265 – 575	0.64/0.77
280 – 535	0.67/0.75

***Table 4**: MAGFLO™ NGS -to-sample ratios used for double-sided size selection.*

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.

Problem	Cause	Solution
Low yield	Insufficient input DNA or inefficient PCR reaction	Increase the input amount of DNA or the number of amplification cycles for PCR and/or further optimize the PCR reaction.
	Smaller product size (bp)	Small DNA or RNA fragments normally give lower yields.
	Ethanol residue interference	During the drying step, remove any liquid from the bottom of the well. Make sure to use fresh 80 % ethanol.
	Magnetic bead loss during the procedure	Increase magnetization time. Aspirate slowly. Make sure the plate or tube fits well on the magnet.
	DNA or RNA remains bound to particles	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 drying time to prevent over drying the beads.
	RNA degradation	Ensure that you avoid RNase contamination to prevent RNA loss.
Primer carryover	Insufficient washing of the magnetic beads	Wash the magnetic beads one more time with 80 % 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.
Double-sided size selection does not give the expected DNA fragment size	Selected DNA fragments are too small	The ratio of MAGFLO™ NGS magnetic beads to the sample volume was too high. Try adding fewer magnetic beads during the size selection process to obtain larger DNA fragments.
	Selected DNA fragments are too large	The ratio of MAGFLO™ NGS magnetic beads to the sample volume was too low. Try adding more magnetic beads during the size selection process to obtain smaller DNA fragments.
	Contamination of larger DNA fragments after size selection	This can be caused by magnetic bead carryover from the first binding step to the second. Avoid transferring magnetic beads after the first binding step.
Problems in downstream applications	Salt carryover	80 % 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 80 % ethanol.

***Table 5**:Troubleshooting guide.*

6 User insights on INTEGRA's benchtop pipetting solutions

6.1 Walk-away solutions for MAGFLO™ NGS magnetic beads

If you are interested in a walk-away workflow, please refer to the automated workflow set-up on the ASSIST PLUS with an integrated magnetic separation module (MAG/HEATMAG). A detailed script with optimized pipetting parameters is available for downloading in the application note on our website.

Note: If you want to modify the program, please refer to the handout to change the sample input and the elution volume in VIALAB software, available on request from your regional INTEGRA sales representative.

***Figure 3**: ASSIST PLUS pipetting platform with an integrated MAG module and the VOYAGER pipette from INTEGRA.*

7 Ordering information

Contact your regional INTEGRA sales representative to place an order.

Article number	Description	Number of reactions for single sided size selection*	Number of reactions for double sided size selection **
7000	MAGFLO™ NGS magnetic beads, 1 ml	~55	~25
7002	MAGFLO™ NGS magnetic beads, 50 ml	~2'777	~1'250
7004	MAGFLO™ NGS magnetic beads, 500 ml	~27'777	~2'500

***Table 6**: Part numbers available for MAGFLO™ NGS magnetic beads.*

* *Number of reactions is based on a 10 µl reaction volume (e.g. for a bead cleanup 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).*

** *Number of reactions is based on a 50 µl reaction volume, with 40 µl magnetic beads used per reaction.*

8 Disclaimer

Every effort has been made to provide complete and accurate information in this manual. Although this manual should contain a specifically labeled warranty notice for the product, INTEGRA Biosciences AG makes no representations or warranties with respect to the contents of this manual and reserves the right to change this manual without notice if and when improvements are made.

INTEGRA Biosciences AG shall not be liable for any loss, damage, repair costs, incidental or consequential damages of any kind, whether based on express or implied warranty, contract, omission or strict liability, arising in connection with the design, development, installation or use of the products.

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 137960, the version is V02.

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.

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