INSTRUCTION MANUAL

Monarch® Mag PCR & DNA Cleanup Kit (5 µg)

NEB #T4130V/S/L

10/100/400 preps

Version 1.0 10/25

Table of Contents

Kit Contents and Storage

Component	NEB #	Application/Usage	T4130V 10 preps	T4130S 100 preps	T4130L 400 preps	Storage Temperature
Monarch Buffer BZ	T1114	Binding buffer concentrate (1.42X)	8.4 ml	42 ml x 2	168 ml x 2	15-25°C
Monarch Buffer WZ	T1115	Wash buffer concentrate (5X)	5 ml	12 ml	26 ml x2	15-25°C
Monarch Buffer EY	T1116	Elution buffer	3 ml	25 ml	45 ml	15-25°C
Monarch Mag Beads M2	T1157	Magnetic beads for nucleic acid purification	0.45 ml	2.2 ml	9 ml	4°C upon opening

Storage Recommendation

- All buffers should be stored at room temperature.
- See individual component labels for specific storage guidance.
- Monarch Mag Beads M2 should be stored at 4°C upon opening.
- Always keep reagent bottles tightly closed.

Intended Use

The Monarch Mag PCR & DNA Cleanup Kit (5 µg) is developed for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Safety Information

- Monarch Buffer BZ contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. Do not add bleach or acidic solutions directly to the buffers or sample preparation waste.
- For more information regarding the composition of buffers, please consult the Safety Data Sheets available on our website www.neb.com/T4130.
- Proper laboratory safety practices should be employed using this kit, including the use of lab coats, gloves, and eye protection.

Quality Control

To help ensure consistent quality and performance, each lot of this kit is tested for predetermined quality control and functional specifications.

Introduction

The Monarch Mag PCR & DNA Cleanup Kit (5 µg) is designed for the rapid and consistent purification and concentration of up to 5 µg of DNA (both double- and single-stranded) from common enzymatic reactions, including restriction digestion, ligation, reverse transcription, PCR, and rolling circle amplification (RCA). The streamlined bind-wash-elute workflow, using silica-coated magnetic beads, yields highly pure DNA that is free of inhibitors and ready for immediate use in downstream applications. Flexible by design, the kit supports both manual and automated workflows and can be readily scaled to accommodate varying binding capacity needs.

Features of this kit include:

- **High Performance:** Recover up to 5 µg of DNA with high yield and purity. Effectively remove primers, detergents, nucleotides, DMSO, betaine, and other low-molecular-weight reaction components.
- **Reliability:** Consistent results for purification and cleanup.
- **Flexible Workflow:** Compatible with both manual and automated processing, using silica-coated magnetic beads.
- **Scalability:** Easily adapted to different binding capacity requirements.
- **Broad Sample Compatibility:** Purify a wide range of fragment sizes, from very small (< 50 bp) oligonucleotides to large (> 25 kb) DNA fragments, using the provided modified protocols.
- **Application Ready:** Purified DNA is suitable for downstream molecular biology applications, including restriction digestion, ligation, amplification, sequencing, and *in vitro* transcription.

Sustainability and Recycling Information

Monarch DNA and RNA Purification kits are designed for sustainability and performance. Learn more about Monarch sustainability at www.neb.com/monarchsustainability.

- **Sustainable performance**: Monarch kits use significantly less plastic in spin columns, buffer bottles, and other plastic components than leading alternatives. Magnetic bead formats provide a low-plastic option to traditional spin columns.
- **Flexible purchasing options:** Monarch kit components such as magnetic beads, spin columns, and buffers are available individually to suit your workflow needs.
- **Same performance, design and formulations:** Standalone products are the same components and formulations that are included in complete kits.
- **Streamlined packaging:** Monarch kits come in sturdy, right-sized reusable boxes and include quick protocol cards for easy reference.
- **Sustainable and recyclable packaging**: Monarch kits are printed with minimal ink using eco-friendly practices and renewable energy. Kit boxes, inserts and paper materials use recycled and recyclable paper.

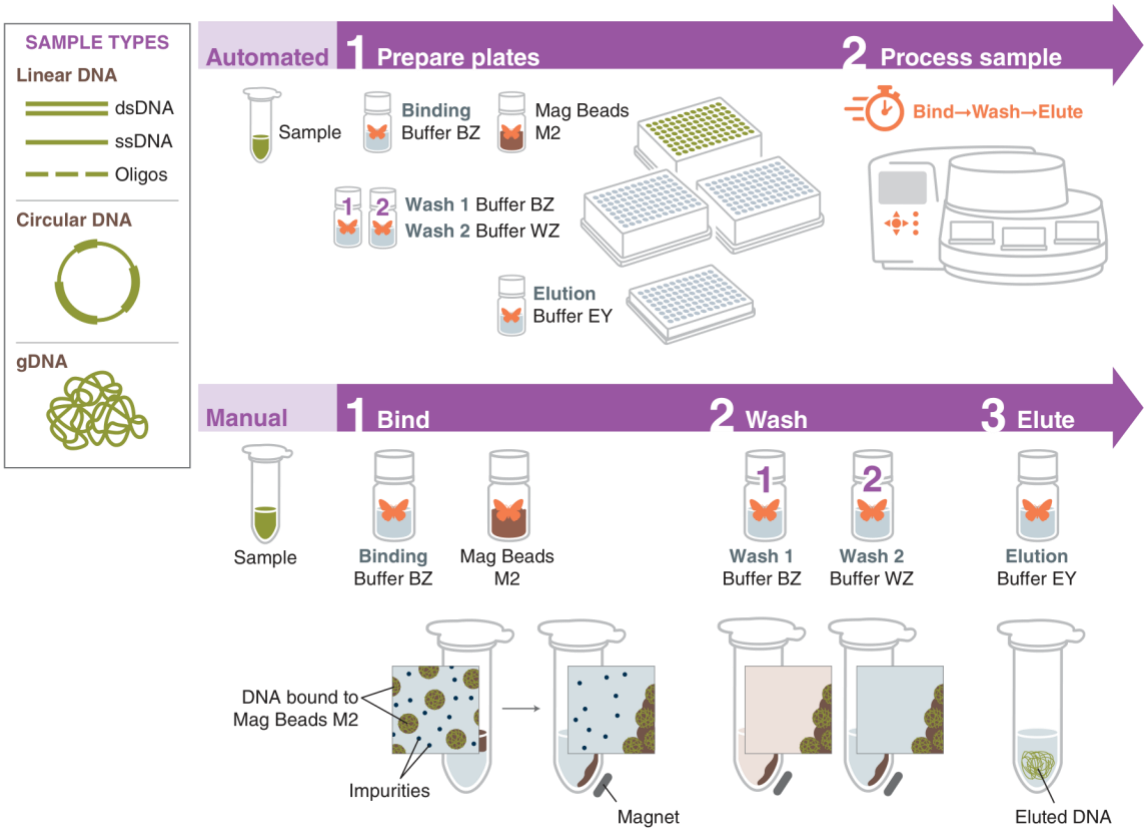
Help keep Monarch sustainable by recycling after use. Learn more about how to recycle Monarch boxes and kit components at www.neb.com/monarchrecycling.

Background

The Monarch Mag PCR & DNA Cleanup Kit (5 µg) features a streamlined bind–wash–elute workflow utilizing silica-coated magnetic bead technology for efficient DNA purification. The process is supported by optimized buffers that enable selective and high-yield DNA recovery. Monarch Buffer BZ, formulated with high salt concentrations, promotes optimal and specific binding of DNA to the magnetic beads. Once binding is complete, a fresh aliquot of Monarch Buffer BZ (Wash 1) is used to remove proteins and nonspecifically bound biomolecules. Monarch Buffer WZ (Wash 2) is then applied to eliminate any residual enzymes, salts, detergents, and other reaction components while DNA remains bound to the magnetic beads. Finally, DNA is eluted under low-salt conditions, resulting in highly pure DNA suitable for a wide range of downstream applications, including restriction digestion, DNA sequencing, ligation, *in vitro* transcription reaction templates, and other enzymatic processes.

The kit supports both manual and automated workflows, enabling consistent, reliable, and high-throughput purification.

Figure 1: DNA Cleanup Workflow



Properties

Purification Format	Magnetic bead
DNA Sample Type Compatibility*	DNA from various enzymatic reactions (restriction digestion, ligation, and reverse transcription, PCR, RCA, etc.)
	dsDNA, ssDNA, circular/linear DNA, oligonucleotides, gDNA and RCA
Typical Recovery	70-90 %
DNA Purity	$A_{260/280} > 1.8$ and $A_{260/230} > 1.8$
Binding Capacity	Up to 5 µg using a standard protocol. The binding capacity can be scaled up or down as needed to accommodate different sample requirements.**
Elution Volume	25-50 µl
DNA Size Range	Standard cleanup protocol: 50 bp – 25 kb
	Oligonucleotide cleanup protocol**: ssDNA ≥ 18 nt and dsDNA ≥ 14 bp
	gDNA cleanup protocol.** ≥ 25 kb
Compatible Downstream Applications	Ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing, *in vitro* transcription template

*See the next table for the extended list of DNA samples that can be used with this kit
** Visit the product webpage to find the detailed protocol

Applications & Usage

Listed are selected examples of applications and usage. To see an updated list or related protocol guidance, refer to the product webpage.

Reaction Sample types	PCR cleanup	DNA from PCR reactions can be purified after amplification to remove polymerases, primers, detergents, dNTPs, etc.
	Enzymatic reaction cleanup	Restriction enzymes and modifying enzymes such as ligases, kinases, nucleases, phosphatases are efficiently removed, allowing for effective desalting and concentration of the DNA sample.
	cDNA cleanup	DNA/RNA complexes can be purified post-reverse transcription/ amplification to enable removal of the RT and polymerase as well as nucleotides.
	Labeling cleanup	Unincorporated radiolabeled or fluorescently labeled nucleotides can be removed from the DNA substrate.
	Plasmid cleanup	Plasmid preps from unknown sources may contain inhibitors and unwanted contaminants. Purification and concentration can be easily achieved using this kit.
DNA Types	Oligonucleotide and ssDNA Purification	ssDNA oligonucleotides (≥ 12 nt) and dsDNA fragments (≥ 10 bp) can be purified using the supplemental protocol provided on the product webpage.
	Genomic DNA, DNA size > 25 kb	gDNA and DNA size >25 kb can be purified using supplemental protocol provided on the product webpage.
	Rolling circle amplification (RCA) reaction cleanup	RCA reaction can be purified using supplemental protocol

Important notes before you begin

The Monarch Mag PCR & DNA Cleanup Kit (5 µg) is designed to ensure optimal DNA yield and quality, accommodating various DNA types and sizes. Although the kit is optimized for a broad range of conditions, the following factors should be considered to ensure high-quality results and maximize recovery.

DNA Size

Typically, longer DNA exhibits a stronger affinity to silica in the presence of chaotropic salt, resulting in tight binding. For Monarch Mag PCR & DNA Cleanup Kit (5 µg), DNA fragments larger than 15 kb may bind tightly to the silica matrix, making elution more difficult. If working with DNA longer than 15 kb, a modified elution method can be employed to increase elution efficiency. For a more detailed procedural guide, we recommend reading the full protocol provided in this manual or on our product webpage.

For DNA size < 50 bp (oligonucleotides), > 25 kb (gDNA) or RCA reaction purifications, we provide a separate protocol for optimal recovery. Please visit our product webpage for detailed procedural guidance.

Scaling purification for various sample volumes and binding capacity

The purification workflow can be scaled by adjusting buffer volumes and bead amounts in proportion to the sample size. For small volumes (≤ 100 µl), standard 1.5–2.0 ml microcentrifuge tubes are sufficient. For larger sample volumes, higher-capacity vessels such as conical tubes (> 5 ml) may be required to accommodate binding and mixing. Selecting appropriately sized vessels ensures consistent performance across varying sample volumes.

Binding capacity is determined by the number of beads used per prep. Increasing the number of beads expands DNA binding capacity, while reducing the number of beads conserves reagents when working with low DNA inputs. When scaling beyond the standard bead volume provided in the kit (20 µl, for up to 5 µg DNA), additional components (e.g., beads, buffers, wash solutions) may be required. Confirm that sufficient reagents are available before starting purification.

For high-throughput automated workflows, typically PCR plates or deep-well plates are recommended. Always confirm that the selected vessels are compatible with the instrument and that plate capacity is sufficient for the intended buffer and sample volumes.

For convenience, example protocols for scaling up or down are available on the product webpage.

Procedure Notes

Storing and Handling of Monarch Mag Beads M2

Monarch Mag Beads M2 should be stored at 4°C when not in active use. Before use, always allow the beads to warm to room temperature and mix thoroughly, as they can settle quickly.

While bead performance is consistent, incomplete resuspension may reduce binding efficiency and lead to inconsistent results. During purification and magnetic separation, always allow sufficient time for complete pelleting, and remove supernatants carefully to avoid disturbing the pellet.

Binding of DNA to Beads

DNA binds to magnetic beads through interactions with the bead surface in the presence of the binding buffer (Monarch Buffer BZ). Proper mixing during this step is essential for efficient recovery. Using a thermal mixer or consistent gentle agitation (e.g., occasional mixing on a vortex mixer) ensures thorough contact between beads and DNA. Binding typically occurs within 5 minutes; extending incubation to 10 minutes can improve recovery when DNA input approaches maximum binding capacity.

Bead Drying and Elution

Following the final wash, beads should be dried thoroughly to remove residual ethanol, since carryover can interfere with downstream applications. Avoid over-drying, which can reduce elution efficiency and DNA yield; beads that appear cracked or matte light brown indicate over-dried beads. Properly dried beads should appear shiny and uniformly dark.

For elution, DNA is released from the beads using a low-salt buffer or nuclease-free water. Gentle mixing promotes complete recovery, while incubation at 50°C and/or extended mixing can improve recovery of large DNA fragments (>15 kb). Elution volumes may be adjusted based on the desired DNA concentration—smaller volumes yield more concentrated DNA but may reduce total recovery.

For automated workflows, adjust minimum elution volumes according to the specifications and requirements of the system being used.

General Guidelines for Monarch® Mag PCR & DNA Cleanup Kit (5 µg)

- A starting sample volume of 20-100 µl is recommended for the standard DNA Cleanup workflow. For smaller samples, nuclease-free water or TE buffer can be used to adjust the volume to the recommended volume range. Sample volumes larger than 100 µl can be used; however, additional Monarch Buffer BZ may be required. If extra Monarch Buffer BZ is needed, it is available for purchase separately.

Equipment and Reagents Required & Supplied by the User

Equipment

- For manual workflow: magnetic rack or plate appropriate for tube/plate size, microcentrifuge/benchtop mini centrifuge or compatible centrifuge for tube and plate size
- For automated workflow: appropriate hardware for automation instrument (e.g., magnet, shaker, heat block)

Reagents/supplies

- Isopropanol (≥ 99%)
- Ethanol (≥ 95%)
- 1.5 ml microfuge tube or tube/plate appropriate for prep volume
- Optional: Nuclease-free water for elution, if provided elution buffer will not be used

Buffer Preparation

- Monarch Buffer BZ and Monarch Buffer WZ are provided as a concentrate. Please prepare the buffers accordingly using the table below:

	T4130V (10 preps)	T4130S (100 preps)	T4130L (400 preps)
Monarch Buffer BZ Add 0.43 volume of isopropanol per volume of Buffer BZ	Add 3.6 ml of isopropanol	Add 18 ml of isopropanol	Add 72 ml of isopropanol
Monarch Buffer WZ Add 4 volumes of ethanol (≥ 95%) to one volume of Buffer WZ	Add 20 ml of ethanol	Add 48 ml of ethanol	Add 104 ml of ethanol

- Monarch Buffer EY is provided at 1x concentration, no additional preparation is needed
- Always keep all buffer bottles tightly closed when not in use
- Store kit components at the recommended storage conditions on component labels and in the product manual

Protocols

1. Manual Workflow for Standard PCR & DNA Cleanup Protocol
2. Automated workflow guidance for PCR & DNA Cleanup Protocol

Manual Workflow for Standard PCR & DNA Cleanup Protocol

1. **Add 5 volumes of Monarch Buffer BZ (ensure isopropanol has been added) to 1 volume of sample. Mix well.** A sample volume of 20-100 µl is recommended. The provided Buffer BZ is sufficient for sample volumes up to 100 µl. For larger sample volumes, additional Buffer BZ may be required (available separately).

2. **Thoroughly mix Monarch Mag Beads M2 and add 20 µl of bead suspension to the sample. Mix well for 5-10 minutes to allow binding. Spin down briefly to collect liquid.** The bead solution settles quickly. Mix the bead solution well before adding. For consistent binding, mixing samples at approximately 1400 rpm on a thermal mixer (or a similar instrument) is recommended; however, occasional vortexing can also be used as an alternative. While 5 minutes of mixing is generally sufficient, extending the incubation to 10 minutes is recommended to maximize recovery.

3. **Separate the beads using a magnet for 1-2 minutes or until the beads have fully pelleted. Carefully remove and discard the supernatant without disturbing the bead pellet.** The pellet will form along the wall of the tube or plate. The supernatant should appear clear once the beads are fully separated.

4. **Wash by adding 500 µl Monarch Buffer BZ (Wash 1). Vortex briefly to mix, then spin down to collect beads and buffer.**

5. **Separate the beads using a magnet for 1-2 minutes or until the beads have fully pelleted. Carefully remove and discard the supernatant without disturbing the bead pellet.** The pellet will form along the wall of the tube or plate. The supernatant should appear clear once the beads are fully separated.

6. **Wash by adding 500 µl Monarch Buffer WZ (Wash 2). Vortex briefly to mix, then spin down to collect beads and buffer.**

7. **Repeat Step 5.**

8. **Air-dry the beads at room temperature for up to 10 minutes.** Beads typically dry within 2-3 minutes. The bead pellet should still appear shiny with no visible liquid. Avoid over-drying, as this will significantly reduce DNA yield. Properly dried beads should appear shiny and dark brown, without cracks or a matte light brown appearance.

9. **Elute DNA by adding 25-50 µl Monarch Buffer EY and mixing for 2 minutes.** For consistency, mixing at 1400 rpm on a thermal mixer (or similar instrument) is recommended. Alternatively, occasional vortexing can be used. Ensure no beads remain on the side of the tube. For large DNA fragments (≥ 15 kb), incubate at 50-55°C for 5 minutes to maximize recovery.

10. **Separate the beads using a magnet for 1-2 minutes or until the beads have fully pelleted. Carefully transfer the eluted DNA to a new tube or plate.**

Automated Workflow Guidance for PCR & DNA Cleanup Protocol

General guidance for automated DNA Cleanup

Monarch Mag PCR & DNA Cleanup Kit (5 µg) is compatible with automated platforms. Refer to the product webpage for the most up-to-date information on additional guidance and protocols.

I: Prepare the instrument, buffers and samples

- Ensure the automation instrument is equipped with the appropriate hardware (e.g., magnet, shaker, heat block)
- Prepare all necessary reagents and consumables

II: Sample preparation

- If the automation instrument does not support liquid handling, perform sample preparation manually before loading onto the instrument

1. Add 5 volumes of Monarch Buffer BZ to 1 volume of the sample. Mix well.

2. Add 20 µl of Monarch Mag Beads M2 to the sample. The Monarch Mag Beads M2 settle quickly. Mix the bead solution well just before adding to ensure uniform bead distribution.

III: Binding

1. Mix the sample plate or tubes for 5-10 minutes. Mixing speed and method may vary depending on the platform. Ensure beads remain fully suspended throughout the mixing process.

2. Separate beads using a magnet for 1-2 minutes. Remove supernatant.

IV: Wash

1. Add 500 µl of Monarch Buffer BZ (Wash 1) to the beads and mix well. Mix for 30-60 seconds at a speed that ensures the magnetic beads remain fully suspended in the wash buffer throughout the step. A volume of 500 µl is recommended for deepwell plates; if using labware with different capacities, adjust the volumes accordingly.

2. Separate beads using a magnet for 1-2 minutes. Remove supernatant.

3. Add 500 µl of Monarch Buffer WZ (Wash 2) to beads and mix well. Mix for 30-60 seconds at a speed that ensures the magnetic beads remain fully suspended in the wash buffer throughout the step. A volume of 500 µl is recommended for deepwell plates; if using labware with different capacities, adjust the volumes accordingly.

4. Repeat step 2.

V: Elute

1. Air dry the beads at room temperature. Beads typically dry within 2-3 minutes but drying time may vary by system. Optimize this step for each platform to ensure efficient processing without over-drying.

2. Add 25-50 µl of Monarch Buffer EY to the beads. Mix for 2 minutes at a speed that keeps beads suspended. Elution volume can be adjusted based on automation platform compatibility.

3. Separate/collect beads using a magnet for 1-2 minutes.

4. Transfer the eluate to a new plate or tube.

Troubleshooting

Problem	Common Cause	Suggestions/Solutions
No DNA purified	Ethanol not added to wash buffer	Ensure proper amount of ethanol was added to the wash buffer.
Low DNA yield	Reagent added incorrectly	Check the protocol to confirm that buffers have been properly reconstituted and that the correct volumes are used. Verify that the appropriate buffer is added at each step.
	Too few beads added	Ensure that the Monarch Beads M2 solution is thoroughly mixed before adding to the sample. Because the beads settle quickly, inadequate mixing may result in dispensing fewer beads than required, reducing binding capacity and lowering DNA yield.
	Insufficient binding	Ensure samples with beads are thoroughly mixed. Minimum of 5 minutes of mixing is recommended to ensure sufficient binding of DNA to the beads. If amount of DNA is nearing maximum capacity, 10 minutes of mixing is recommended.
	Beads over-dried	Beads that are over-dried appear matte and light brown, often with visible surface cracks. To prevent DNA loss, proceed to elution once excess liquid has been removed and the beads still appear shiny, rather than allowing beads to over-dry.
	Incomplete elution	Ensure that Monarch Buffer EY (elution buffer) or nuclease-free water is added and that the bead pellet is thoroughly resuspended. Mix well for 2 minutes. Using larger elution volumes or extending the incubation time can improve DNA recovery, particularly when working near the maximum binding capacity or with large DNA fragments (>15 kb). Alternatively, heating the beads and elution buffer to 50–55°C during mixing can further increase yield.
	Bead lost during prep	Use a strong magnet to ensure effective bead separation, and carefully remove the supernatant without disturbing the bead pellet, as any beads removed with the supernatant may result in DNA loss.
Low DNA purity and performance	Ethanol is carried over from insufficient wash buffer removal and/or bead drying	Ensure beads are sufficiently dried without excess liquid. The bead should look still shiny without free-flowing liquid. Removing as much wash buffer as possible after wash 2 step can improve drying time.
	Trace amounts of salt carried over	Carried-over salts will be indicated by a low $A_{260/230}$ ratio. Ensure wash buffers are removed as much as possible and proper drying of the beads.
	Beads carried over	Use a strong magnet that is appropriately sized for the volume and vessel used. Separate the beads for 1–2 minutes or until they have formed a pellet. If the beads do not separate and pellet within 2 minutes, allow an additional minute to ensure complete pelleting or source a stronger magnet.

For more troubleshooting and FAQs, please visit the product webpage or reach out to our technical support team at info@neb.com

Ordering Information

View the entire Monarch DNA & RNA Purification portfolio at NEBMonarch.com.

Monarch® Mag PCR & DNA Cleanup Kit (5 µg)

PRODUCT	NEB#
Monarch® Mag PCR & DNA Cleanup Kit (5 µg)	T4130
Kit components available individually	
Monarch® Mag Beads M2	T4105
Monarch® Buffer BZ	T1114
Monarch® Buffer WZ	T1115

NEB Companion Products

PRODUCT	NEB#
Monarch® Spin PCR & DNA Cleanup Kit (5 µg)	T1130
Monarch® Spin High-Capacity DNA Cleanup Kit (100 µg)	T1135
Exo-CIP Rapid PCR Cleanup Kit	E1050
Gel Loading Dye, Purple (6x)	B7024
Gel Loading Dye, Purple (6x), no SDS	B7025
Quick-Load Purple® 1 kb DNA Ladder	N0552
Quick-Load Purple® 100 bp DNA Ladder	N0551
Quick-Load Purple® 1kb Plus DNA Ladder	N0550
T4 DNA Ligase	M0202
Blunt/TA Ligase Master Mix	M0367
Instant Sticky-end Ligase Master Mix	M0370
Q5® Hot Start High-Fidelity 2x Master Mix	M0494
One*Taq*® DNA Polymerase	M0480

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	10/25

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