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NEB Monarch® RNA Cleanup Kit

New England Biolabs[1]

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol is for the Monarch RNA Cleanup Kits (NEB #'s #T2030, T2040, and T2050). The protocol can also be used with the Monarch RNA Cleanup Columns (NEB #'s T2037,T2047, andT2057) and associated buffers (NEB #'s T2041,T2042).
The standard protocol outlined below will purify RNA ≥ 25 nt. A simple modification in Step 2 can allow for the purification of RNA as small as 15 nt.

EXTERNAL LINK

https://neb.com/protocols/2018/06/28/monarch-rna-cleanup-kit-protocol

ATTACHMENTS

T2030_T2040_T2050_Quick_Protocol_Card_Monarch_RNA_Cleanup.pdf manualT2030_T2040_T2050.pdf

PROTOCOL CITATION

New England Biolabs 2020. NEB Monarch® RNA Cleanup Kit. **protocols.io** https://protocols.io/view/neb-monarch-rna-cleanup-kit-74dhqs6

EXTERNAL LINK

https://neb.com/protocols/2018/06/28/monarch-rna-cleanup-kit-protocol

KEYWORDS

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OWNERSHIP HISTORY

Oct 10, 2019	Anita Broellochs	protocols.io
Jun 20, 2020	New England Biolabs Tech Support	New England Biolabs
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PROTOCOL INTEGER ID

28517

MATERIALS

NAME	CATALOG #	VENDOR
CW Microfuge Tubes, 1.5ml, 50/pk	AS8201	Promega
Monarch RNA Cleanup	T2030	
Monarch RNA Cleanup Kit (50 ug)	T2040	
Monarch RNA Cleanup Kit	T2050	New England Biolabs

SAFETY WARNINGS

For information regarding the composition of buffers, please consult the Safety Data Sheets available on the NEB website (www.neb.com). Proper laboratory safety practices should be employed, including the use of lab coats, gloves and eye protection.

BEFORE STARTING

Add 4 volumes of ethanol (≥ 95%) to the Monarch RNA Wash Buffer before use, as directed on the bottle.
For the 10-prep kit, add 10 ml of ethanol to 2.5 ml of Monarch RNA Cleanup Wash Buffer. For the 100-prep kit, add 80 ml of ethanol to 20 ml of Monarch RNA Cleanup Wash Buffer.

If a precipitate has formed in the Monarch RNA Cleanup Binding Buffer, warm to room temperature to re-dissolve before use.

1 **Add 100 µl RNA Cleanup Binding Buffer to the 50 µl sample.**

A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume. For samples larger than 50 µl, scale buffer volumes accordingly. Samples with a starting volume > 150 µl will require reloading of the column during Step 3.

When cleaning up large amounts of RNA (> 100 µg, NEB #T2050), some precipitation may occur following the addition of the Monarch RNA Cleanup Binding Buffer and ethanol to the sample (Steps 1 and 2). A pellet containing the RNA of interest may form on the side of the column following the first binding spin (Step 3). To maximize recovery of this RNA, a second elution (Step 7) is recommended.

2 

Add 150 µl ethanol (≥95%) to your sample and mix by pipetting or flicking the tube. Do not vortex.

This will enable the binding of RNA ≥ 25 nt. If you wish to bind RNA as small as 15 nt, add 2 volumes (300 µl) of ethanol to your sample instead of 1 volume (150 µl). The addition of 2 volumes of ethanol shifts the cutoff size of RNA binding from 25 nt down to 15 nt.

3 

Insert column into collection tube, load sample onto column and close the cap. Spin for 00:01:00 at 16000 rpm , then discard flow-through.

For diluted samples > 900 µl, load a portion of the sample, spin, and then repeat as necessary.

To save time, spin for 30 seconds, instead of 1 minute.

4

Re-insert column into collection tube. Add 500 µl RNA Cleanup Wash Buffer and spin for 00:01:00 at 16000 rpm . Discard the flow-through.

To save time, spin for 30 seconds, instead of 1 minute.

5

Repeat wash: Re-insert column into collection tube. Add 500 µl RNA Cleanup Wash Buffer and spin for 00:01:00 at 16000 rpm . Discard the flow-through.

To save time, spin for 30 seconds, instead of 1 minute.

6 **Transfer column to an RNase-free 1.5 ml microfuge tube (not provided).**

Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.

7

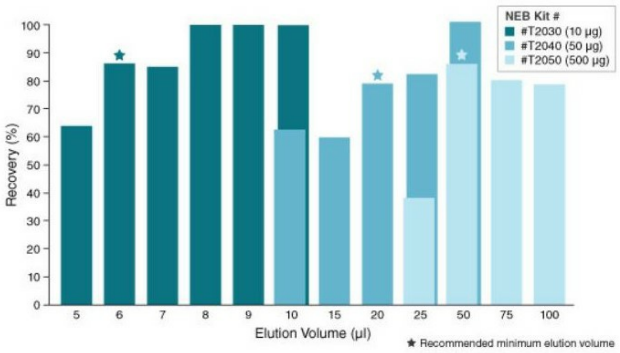
Elute in nuclease-free water at 16000 rpm according to the table below.

The eluted RNA can be used immediately or stored at -70°C. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.

Kit	Elution Volume	Incubation Time	Spin Time
T2030	6-20 µl	N/A	1 minute
T2040	20-100 µl	N/A	1 minute
T2050**	50-100 µl	5 minutes (room temp.)	1 minute

** Yield may slightly increase if a larger volume is used, but the RNA will be less concentrated.

To save time, spin for 30 seconds, instead of 1 minute.



rRNA (10, 50 or 500 µg, respectively of 16S and 23S Ribosomal Standard from E. coli, Sigma) was purified using a Monarch RNA Cleanup Kit (10 µg, NEB #T2030) (50 µg, NEB #T2040) (500 µg, NEB #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting A_{260} as measured using a Trinean DropSense 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch RNA Cleanup Kit (500 µg, NEB #T2050).

Recovery of RNA from Monarch RNA Cleanup Kits with Varying Elution Volumes