Jun 22, 2020

# Purification of RNA from the Aqueous Phase Following TRIzol®/Chloroform Extraction using the Monarch® RNA Cleanup Kits

New England Biolabs[1]

[1]New England Biolabs

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

New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

Isabel Gautreau
New England Biolabs

ABSTRACT

RNA isolation reagents containing guanidine thiocyanate and phenol (e.g., TRIzol, RNAzol®, QIAzol®, etc) combined with chloroform extraction, are often used for sample lysis and RNA purification. The aqueous phase from any guanidinium thiocyanate-phenol-chloroform extraction can be cleaned up using the Monarch RNA Cleanup Kits (NEB #T2030, T2040, T2050), thereby eliminating the need for tedious RNA precipitation step

EXTERNAL LINK

https://neb.com/protocols/2018/06/28/purification-of-rna-from-the-aqueous-phase-following-trizol-chloroform-extraction-using-the-monarch-rna-cleanup-kits

PROTOCOL CITATION

New England Biolabs 2020. Purification of RNA from the Aqueous Phase Following TRIzol®/Chloroform Extraction using the Monarch® RNA Cleanup Kits. **protocols.io** https://protocols.io/view/purification-of-rna-from-the-aqueous-phase-followi-7rxhm7n

EXTERNAL LINK

https://neb.com/protocols/2018/06/28/purification-of-rna-from-the-aqueous-phase-following-trizol-chloroform-extraction-using-the-monarch-rna-cleanup-kits



CREATED

Sep 27, 2019

LAST MODIFIED

Jun 23, 2020

OWNERSHIP HISTORY

| | | |
|---|---|---|
| Sep 27, 2019 | Anita Broellochs | protocols.io |
| Jun 20, 2020 | New England Biolabs Tech Support | New England Biolabs |
| Jun 23, 2020 | Isabel Gautreau | New England Biolabs |

PROTOCOL INTEGER ID

28183

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

- Add 4 volumes of ethanol (≥ 95 %) to the Monarch RNA Wash Buffer before use, as directed on the bottle.
- All centrifugation steps should be carried out at 16,000 x g. (~ 13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

1 

Following guanidinium-thiocyanate-phenol-chloroform extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided).

2 

Add 1 volume of ethanol (≥ 95 %).

3 

Mix well by pipetting up and down or flicking the tube. **Do not vortex.**

4 

Insert an RNA cleanup column into a collection tube, load sample onto the column and close the cap.

5 Spin for **00:01:00** , 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.

6 

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

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

7 

Repeat wash (Step 6).

8 

Transfer the 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.

9 Elute in nuclease-free water 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 center of the matrix and not the wall of the column to maximize elution efficiency.

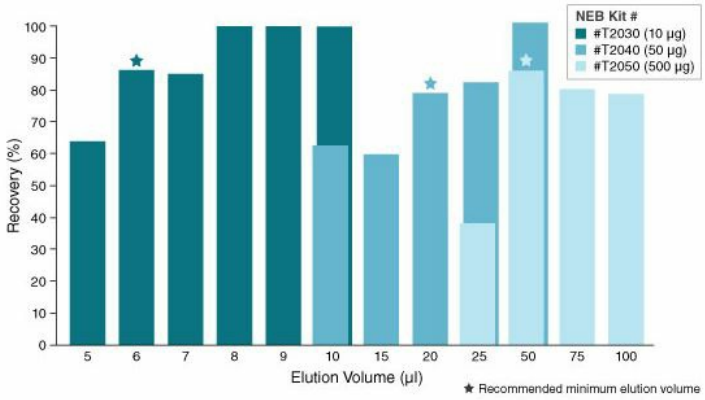
| KIT | ELUTION VOLUME | INCUBATION TIME | SPIN TIME |
|---|---|---|---|
| T2030 | 6 – 20 µl | N/A | 1 minute |
| T2040 | 20 – 100 µl | N/A | 1 minute |
| T2050** | 50 – 100 µl | 5 minutes (Room temp.) | 1 minute |

* 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– 3). A pellet containing the RNA of interest may form on the side of the column following the first binding spin (Steps 4 and 5). To maximize recovery of this RNA, a second elution is recommended.

** 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.

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




*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).*