Version 3

Nov 30, 2020

Quick Protocol for Monarch® Total RNA Miniprep Kit (NEB #T2010) V.3

New England Biolabs[1]

[1]New England Biolabs

1 *Works for me* dx.doi.org/10.17504/protocols.io.bp9cmr2w

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ABSTRACT

Quick Protocol for Monarch® Total RNA Miniprep Kit (NEB #T2010).

Quickly and easily purify up to 100 µg of high-quality total RNA from multiple sample types – all with one kit!

- For use with blood, cells and tissues
- Also works with tough to lyse samples (bacteria, yeast, plant)
- Effectively purifies total RNA of all sizes, including small RNAs >20 nt
- Efficient genomic DNA removal (column and DNase I-based)
- Contains Proteinase K for processing of tissues and blood samples
- Includes RNA Protection Reagent for sample preservation
- Excellent value
- Kit components available separately

EXTERNAL LINK

https://www.neb.com/protocols/2017/11/28/quick-protocol-for-monarch-total-rna-miniprep-kit-neb-t2010

ATTACHMENTS

T2010_Monarch_RNA_Kit_072817.pdf manualT2010.pdf

DOI

dx.doi.org/10.17504/protocols.io.bp9cmr2w

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PROTOCOL CITATION

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EXTERNAL LINK

https://www.neb.com/protocols/2017/11/28/quick-protocol-for-monarch-total-rna-miniprep-kit-neb-t2010

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ATTACHMENTS

T2010_Monarch_RNA_Kit_072817.pdf manualT2010.pdf

GUIDELINES

We recommend that first-time users of this kit review the product manual before starting. The manual provides additional relevant information to consider at various steps. This quick protocol is meant for experienced users.

RNA Purification Consists of Two Stages:
PART 1: Sample Disruption and Homogenization (Differs depending on starting material)
PART 2: RNA Binding and Elution

Have any questions?
Our tech support scientists would be happy to help. Email us at info@neb.com.

MATERIALS TEXT

MATERIALS

Monarch Total RNA Miniprep Kit **New England Biolabs Catalog #T2010S**

SAFETY WARNINGS

Please refer to the SDS for safety warnings.

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Quick Protocol for Monarch® Total RNA Miniprep Kit (NEB #T2010).

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BEFORE STARTING

- Please review the important information under the “Guidelines" & "Warnings” tabs before beginning.
- Addition of RNA Lysis Buffer and all subsequent steps should be performed at room temperature to prevent formation of precipitate. If samples are accidentally placed on ice and precipitate forms, allow the samples to return to room temperature to resolubilize before loading onto the column.
- Protocols are also available for RNA reaction cleanup, RNA fractionation, and extraction of RNA from other sample types (including those in preservation reagents or TRIzol®).

For the 50 prep kit:

- Reconstitute DNase I by adding 275 µl nuclease-free water. Gently invert to mix. Aliquot for storage at -20°C to

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minimize freeze-thaw cycles.

- Reconstitute Proteinase K (Prot K) by adding 1040 µl of Proteinase K Resuspension Buffer. Vortex and store at -20°C.
- Add 100 ml ethanol (≥ 95%) to the 25 ml RNA Wash Buffer concentrate.

PART 1: Sample Disruption and Homogenization

1 Please select your starting material of the following:
- **Cultured Mammalian Cells**
- **Mammalian Whole Blood (Fresh or Frozen)**
- **Tissue or Leukocytes**
- **Tough-to-Lyse Samples (bacteria, yeast, plant, etc.) using Mechanical Lysis**

Step 1 includes a Step case.
Cultured Mammalian Cells
Mammalian Whole Blood (Fresh or Frozen)
Tissue or Leukocytes
Tough-to-Lyse Samples

step case

Cultured Mammalian Cells

2 Pellet cells by centrifugation (500 x *g*) for 1 min.
00:01:00 Centrifugation

3 Discard supernatant.

4 Resuspend pellet in RNA Lysis Buffer (according to table below) by pipetting gently to avoid foaming.

AMOUNT OF CELLS	VOLUME OF RNA LYSIS BUFFER
up to 3 x 10^6	300 µl
3 x 10^6 to 1 x 10^7	≥ 600 µl

Do not place samples on ice. For frozen pellets, thaw briefly before resuspension.

PART 2: RNA BINDING AND ELUTION

5 Transfer up to 800 µl of the sample from PART 1 to a gDNA removal column

(light blue) fitted with a collection tube.
For sample identification, **label collection tubes**, as gDNA removal columns will be discarded after spinning.

For sample volumes > 800 µl (column reservoir capacity), columns may be reloaded.

All centrifugation steps should be performed at 16,000 x g.

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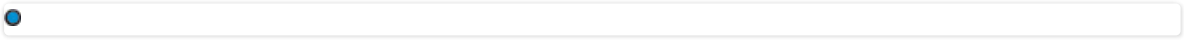
6 Spin for 30 seconds to remove most of the gDNA. **SAVE THE FLOW-THROUGH** (RNA partitions here). Discard the gDNA removal column.

00:00:30 Spinning

7 Add an equal volume of ethanol (≥ 95%) to the flow-through and mix thoroughly by pipetting. **Do not vortex.** To exclude RNA ≳ 200 nt, add only 1/2 volume ethanol to flow-through.

8 Transfer mixture to an RNA purification column



(dark blue) fitted with a collection tube. Spin for 30 seconds. Discard flow-through. If further gDNA removal is essential for downstream applications, proceed to on-column DNase I treatment, steps 9-11 (recommended). If not, proceed to **Step 12**.

00:00:30 Spinning

9 **[Optional (but recommended)]** On-column DNase I treatment for enzymatic removal of residual gDNA: Add 500 µl RNA Wash Buffer and spin for 30 seconds. Discard flow-through.

500 µl RNA Wash Buffer

00:00:30 Spinning

10 **[Optional (but recommended)]** In an RNase-free microfuge tube (not included), combine 5 µl DNase I with 75 µl DNase I Reaction Buffer and pipet directly to the top of the column matrix.

5 µl DNase I

75 µl DNase I Reaction Buffer

11 **[Optional (but recommended)]** Incubate for 15 minutes at room temperature.

00:15:00 Incubation

12 Add 500 µl RNA Priming Buffer and spin for 30 seconds. Discard flow-through.

500 µl RNA Priming Buffer

00:00:30 Spinning

13 Add 500 µl RNA Wash Buffer and spin for 30 seconds. Discard flow-through.

500 µl RNA Wash Buffer

00:00:30 Spinning

14 Add another 500 µl RNA Wash Buffer and spin for 2 MINUTES.

500 µl RNA Wash Buffer

00:02:00 Spinning

15 Transfer column to an RNase-free microfuge tube (not included). Use care to ensure the tip of the column does not contact the flow-through. If in doubt, re-spin for 1 minute to ensure no ethanol is carried over.

00:01:00 Re-spinning

16 Add 30-100 µl Nuclease-free Water directly to the center of column matrix and spin for 30 seconds.

00:00:30 Spinning

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For best results, elute with at least 50 µl, which is the minimum volume needed to wet the membrane. Lower volumes can be used but will result in lower recovery (elution in 30 µl results in > 80% recovery and 100 µl provides maximum recovery). For spectrophotometric analysis of eluted RNA, it may be necessary to re-spin eluted samples and pipet aliquot from top of the liquid to ensure that the $A_{260/230}$ is unaffected by possible elution of silica particles.

17 Place RNA on ice if being used for downstream steps, at **-20°C** for **short-term storage** (less than 1 week), or at **-80°C** for **long-term storage**. Addition of EDTA to 0.1-1.0 mM may reduce the activity of any contaminating RNases.

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