Monarch® Total RNA Miniprep Kit Protocol Card

NEB #T2010

We recommend that first-time users of this kit review the product manual at neb.com/T2010 before starting. The manual provides additional relevant information to consider at various steps. This quick protocol is meant for experienced users. The website and manual also contain protocols for RNA extraction from other sample types including bacteria, yeast, plant, TRIzol®-extracted samples and samples stored in RNAlater®.

BEFORE YOU BEGIN:

- Reconstitute DNase I by adding 275 µl nuclease-free water. Gently invert to mix. Aliquot for storage at -20°C to 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.
- 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.
- Please refer to product manual at neb.com/T2010 to find protocols for RNA reaction cleanup, RNA fractionation, and extraction of RNA from other sample types (including those in preservation reagents or TRIzol).

RNA PURIFICATION CONSISTS OF TWO STAGES:

PART 1: Sample Disruption and Homogenization

PART 2: RNA Binding and Elution

continued on inside →

PART 1: SAMPLE DISRUPTION AND HOMOGENIZATION

Please follow the protocol specific to your starting material:

❑ Cultured Mammalian Cells:

1. **Pellet cells by centrifugation (500 x *g*) for 1 min. Discard supernatant.**
2. **Resuspend pellet in RNA Lysis Buffer (according to table below) by pipetting gently to avoid foaming. Do not place samples on ice.** For frozen pellets, thaw briefly before resuspension.

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

3. **Proceed to Step 1 of PART 2: RNA Binding and Elution.**

❑ Mammalian Whole Blood (Fresh or Frozen):

1. **Add an equal volume (up to 200 µl) of DNA/RNA Protection Reagent (2X concentrate) to an aliquot of whole blood and vortex briefly. Do not place samples on ice.** For frozen samples, quickly thaw in the presence of 2X DNA/RNA Protection Reagent while vortexing or shaking.
2. **For every 400 µl of DNA/RNA Protection Reagent/blood mixture, add 10 µl of Prot K. Vortex briefly and incubate at room temperature for 30 min.**
3. **Add an equal volume of isopropanol (not included) and vortex briefly. Proceed to Step 4 of PART 2: RNA Binding and Elution.**

❑ Tissue or Leukocytes:

1. **Determine the amount of 1X DNA/RNA Protection Reagent that you will need according to the table below. Prepare the 1X aliquot by diluting the 2X stock with nuclease-free water (not included for this step).**

SAMPLE INPUT AMOUNT	VOLUME 1X DNA/RNA PROTECTION REAGENT
Tissue (up to 10 mg)	300 µl
Tissue (10–30 mg)	300–600 µl
Tissue (30–50 mg)	≥ 600 µl
Leukocytes (up to 3 x 10^6)	300 µl
Leukocytes (3 x 10^6 to 1 x 10^7)	≥ 600 µl

2. **Add 1X DNA/RNA Protection Reagent to sample.** Solid tissue samples should be submerged in protection reagent, not to exceed 10% (w/v). For maximal RNA recovery, tissues can be mechanically homogenized using a bead mill or similar device.
3. **For every 300 µl of DNA/RNA Protection Reagent/sample mixture, add 30 µl Prot K Reaction Buffer + 15 µl Prot K*. Vortex briefly and incubate according to the chart below:**

SAMPLE TYPE	INCUBATION TIME AT 55°C**
Homogenized Tissues	5 min
Solid Tissues	5–30 min
Leukocytes	30 min

* *Doubling the Prot K volume may increase RNA yield for some tissues.*

** *Longer incubation times may result in decreased RNA integrity. Optimal time should be determined empirically, as yield and integrity vary with incubation time.*

Tissue or Leukocytes (Continued):

4. **Vortex sample briefly and spin for 2 min (16,000 x *g*) to pellet debris. Transfer supernatant to an RNase-free microfuge tube (not included).**
5. **Add an equal volume of RNA Lysis Buffer and vortex briefly. Proceed to Step 1 of PART 2: RNA Binding and Elution.**

❑ Tough-to-Lyse Samples (bacteria, yeast, plant, etc.) using Mechanical Lysis:

Mechanical lysis/homogenization is recommended for tough-to-lyse samples. Alternatively, bacteria and yeast may be lysed enzymatically (refer to manual for details). Some gram-negative bacteria (e.g., *E. coli*) do not require mechanical or enzymatic lysis and can by lysed directly by resuspension in DNA/RNA Protection Reagent; however, yields may be lower.

1. **Determine the amount of 1X DNA/RNA Protection Reagent that you will need according to the table below. Prepare the 1X aliquot by diluting the 2X stock with nuclease-free water (not included for this step).**

SAMPLE INPUT AMOUNT			VOLUME 1X DNA/RNA PROTECTION REAGENT
BACTERIA	YEAST	PLANT	
≤ 5 x 10^7	≤ 5 x 10^6		400 µl
> 5 x 10^7–1 x 10^9	5 x 10^6–5 x 10^7	≤ 100 mg	800 µl

2. **Add 1X DNA/RNA Protection Reagent to sample.**
3. **Disrupt sample mechanically using a bead mill or similar device.**
4. **Transfer lysate/homogenate to an RNase-free microfuge tube (not included).**
5. **Spin for 2 min (16,000 x *g*) to pellet debris. Transfer supernatant to an RNase-free microfuge tube (not included).**
6. **Add an equal volume of RNA Lysis Buffer and vortex briefly. Proceed to Step 1 of PART 2: RNA Binding and Elution.**

PART 2: RNA BINDING AND ELUTION

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

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

1. **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.
2. **Spin for 30 seconds to remove most of the gDNA. SAVE THE FLOW-THROUGH (RNA partitions here)**. Discard the gDNA removal column.
3. **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.
4. **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 4a–4c (recommended). If not, proceed to step 5.

   Optional (but recommended): On-column DNase I treatment for enzymatic removal of residual gDNA:

   4a) **Add 500 µl RNA Wash Buffer and spin for 30 seconds.** Discard flow-through.

   4b) **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.**

   4c) **Incubate for 15 minutes at room temperature.** Proceed to Step 5.
5. **Add 500 µl RNA Priming Buffer and spin for 30 seconds**. Discard flow-through.

continued on inside →

PART 2: RNA BINDING AND ELUTION *(continued)*

6. **Add 500 µl RNA Wash Buffer and spin for 30 seconds.** Discard flow-through.
7. **Add another 500 µl RNA Wash Buffer and spin for 2 MINUTES. 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.
8. **Add 30-100 µl Nuclease-free Water directly to the center of column matrix and spin for 30 seconds.** 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.
9. **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.

Need to further concentrate your RNA?

Visit **NEBMonarch.com** to learn about our RNA cleanup kits.

Questions?

Our tech support scientists would be happy to help.
Email us at **info@neb.com**

V2.1 – 1.22 #102959

NEW ENGLAND BioLabs® Inc.