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

New England Biolabs

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

Citation: New England Biolabs Quick Protocol for Monarch® Total RNA Miniprep Kit (NEB #T2010). **protocols.io** dx.doi.org/10.17504/protocols.io.nyadfse
Published: 06 Apr 2018

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.

Before start

- 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 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.

Materials

Monarch Total RNA Miniprep Kit T2010S by New England Biolabs

Protocol

PART 1: Sample Disruption and Homogenization

Step 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**

PART 1: Sample Disruption and Homogenization

Step 2 - Cultured Mammalian Cells.

Pellet cells by centrifugation (500 x *g*) for 1 min.

PART 1: Sample Disruption and Homogenization

Step 3 - Cultured Mammalian Cells.

Discard supernatant.

PART 1: Sample Disruption and Homogenization

Step 2 - Mammalian Whole Blood (Fresh or Frozen).

Add an equal volume (up to 200 µl) of DNA/RNA Protection Reagent (2X concentrate) to an aliquot of whole blood and vortex briefly.

NOTES

Danielle Freedman 21 Mar 2018

Do not place samples on ice. For frozen samples, quickly thaw in the presence of 2X DNA/RNA Protection Reagent while vortexing or shaking.

PART 1: Sample Disruption and Homogenization

Step 3 - Mammalian Whole Blood (Fresh or Frozen).

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.

PART 1: Sample Disruption and Homogenization

Step 2 - Tissue or Leukocytes.

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

PART 1: Sample Disruption and Homogenization

Step 3 - Tissue or Leukocytes.

Add 1X DNA/RNA Protection Reagent to sample.

NOTES

Danielle Freedman 21 Mar 2018

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.

PART 1: Sample Disruption and Homogenization

Step 2 - Tough-to-Lyse Samples.

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**	
≤ $5x10^7$	≤ $5x10^6$		400 µl
≤ $5x10^7$-$1x10^9$	≤ $5x10^6$-$5x10^7$	≤ 100 mg	800 µl

PART 1: Sample Disruption and Homogenization

Step 3 - Tough-to-Lyse Samples.

Add 1X DNA/RNA Protection Reagent to sample.

Warnings

Please refer to the SDS for safety warnings.