RNA Extraction from Cells Using the Monarch® Spin RNA Cleanup Kits

Overview

This protocol is for the extraction of total RNA from cultured mammalian cells (e.g., HeLa cells) using the Monarch Spin RNA Cleanup Kits (NEB #T2030, #T2040, and #T2050). The protocol can also be used with the columns for RNA Cleanup (NEB #T2037, #T2047, and #T2057). Note that this protocol does not remove residual DNA and may not be suitable for downstream methods that require removal of gDNA. We do not recommend on-column DNase I treatment with the Monarch RNA Cleanup Kit as it can often result in residual DNase I activity in eluates. Therefore, if contaminating DNA will interfere with downstream applications, we recommend following this RNA extraction protocol with an in-solution treatment with DNase I (NEB #M0303) and additional RNA cleanup using one of the Monarch Spin RNA Cleanup Kits.

Before You Begin:

- Add 4 volumes of ethanol (≥ 95%) to 1 volume of Monarch Buffer WX.
- If a precipitate has formed in the Monarch Buffer BX, warm to room temperature to re-dissolve before use.
- Recommended starting sample is ≤ 3 X 10^6 cells to allow for sufficient lysis.
- All centrifugation steps should be carried out at room temperature at 16,000 x *g* (~13,000 RPM).

Protocol:

1. **Pellet up to 3 X 10^6 cells by centrifugation (500 x *g*) for 1 minute.** Discard supernatant.

2. **Resuspend pellet in 300 µl Monarch Buffer BX by pipetting gently to avoid foaming**. Do not place samples on ice. For frozen pellets, thaw briefly before resuspension.

3. **Add 300 µl (1 volume) of ethanol (≥ 95%) 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 (600 µl) of ethanol to your sample instead of 1 volume. The addition of 2 volumes of ethanol shifts the cutoff size of RNA binding from 25 nt down to 15 nt.

4. **Insert the column into the collection tube, load the sample onto the column and close the cap. Spin for 1 minute, then discard the flow-through.**
   To save time, spin for 30 seconds, instead of 1 minute.

5. **Re-insert the column into the collection tube. Add 500 µl Monarch Buffer WX, spin for 1 minute, then discard the flow-through.**
   To save time, spin for 30 seconds, instead of 1 minute.

6. **Repeat wash (Step 5).**

7. **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.

8. **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 matrix and not the wall of the column to maximize elution efficiency.
   To save time, spin for 30 seconds, instead of 1 minute.

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 Monarch Buffer BX 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 is recommended.

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