Monarch® RNA Cleanup Kit Protocol Card

NEB #T2030, #T2040, and #T2050

To download the full manual or view a detailed protocol (including guidance on RNA gel extraction or fractionation into small and large RNA pools), please visit:

Monarch RNA Cleanup Kit (10 µg): www.neb.com/T2030
Monarch RNA Cleanup Kit (50 µg): www.neb.com/T2040
Monarch RNA Cleanup Kit (500 µg): www.neb.com/T2050

BEFORE YOU BEGIN:

- Add 4 volumes of ethanol (≥ 95%) to one volume of RNA Cleanup Wash Buffer.
- If a precipitate has formed in the RNA Cleanup Binding Buffer, warm to room temperature to re-dissolve before use.
- All centrifugation steps should be carried out at room temperature at 16,000 x *g* (~13,000 RPM).
- The standard protocol outlined below will purify RNA ≥ 25 nt. A simple modification in step 2 can allow for the purification of RNA as small as 15 nt.

PROTOCOL STEPS:

1. **Add 100 µl RNA Cleanup Binding Buffer to the 50 µl sample.** A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume. For samples larger than 50 µl, scale buffer volumes accordingly. Samples with a starting volume > 150 µl will require reloading of the column during step 3.
2. **Add 150 µl (1 volume) of ethanol (≥ 95%) to your sample 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

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15 nt, add 2 volumes (300 µl) of ethanol to your sample instead of 1 volume (150 µl). The addition of 2 volumes of ethanol shifts the cutoff size of RNA binding from 25 nt down to 15 nt.

3. **Insert column into collection tube, load sample onto column and close the cap. Spin for 1 minute, then discard flow-through.** For diluted samples > 900 µl, load a portion of the sample, spin, and then repeat as necessary.
4. **Re-insert column into collection tube. Add 500 µl RNA Cleanup Wash Buffer, spin for 1 minute, then discard the flow-through.**
5. **Repeat step 4.**
6. **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.
7. **Elute in nuclease-free water according to the table below.** The eluted RNA can be used immediately or stored at -70°C.

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, #T2050), some precipitation may occur following the addition of the Monarch RNA Cleanup Binding Buffer 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.*

Questions?

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

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