RNA Clean-Up and Concentration Micro-Elute Kit

Norgen's RNA Clean-Up and Concentration Micro-Elute Kit provides a rapid method for the purification, cleanup and concentration of RNA for NGS Library preparation and excellent processing of up to 45 µg of RNA isolated using different methods including phenol/guanidine-based protocols, and from various upstream enzymatic reactions such as DNase treatment, labeling and *in vitro* transcription. The minimum recommended elution volume is 8 µL, which enables the concentration of small amounts of all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other reaction components such as proteins, RNases and nucleotides, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including end-point or quantitative reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays and next generation sequencing.

Kit Specifications			
Maximum Column Binding Capacity	45 µg	Minimum Elution Volume	8 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)	Time to Complete 10 Purifications	20 minutes
Maximum Amount of starting Material	45 µg of RNA	Average Recovery	≥ 90%

RNA Clean-Up and Concentration Micro-Elute Kit Benefits	
Small elution volumes	Concentration of small amounts of RNA into 8 µL
Concentrate for NGS Library Preparation	Ideal for concentrating RNA samples prior to NGS library preparation
Fast and easy processing	Rapid spin column format allows for the processing of multiple samples in under 20 minutes.
Versatile input volume	Concentrate from larger elution volumes to more manageable elution volumes
Concentrate from various sample types	Ideal for concentrating RNA purified from exosomes, plasma, serum, urine, and other bodily fluids, and any RNA samples initially purified in large volumes
Isolate inhibitor-free RNA	Suitable for all sizes of RNA, including microRNA (miRNA) without bias

RNA Clean-Up and Concentration Micro-Elute Kit

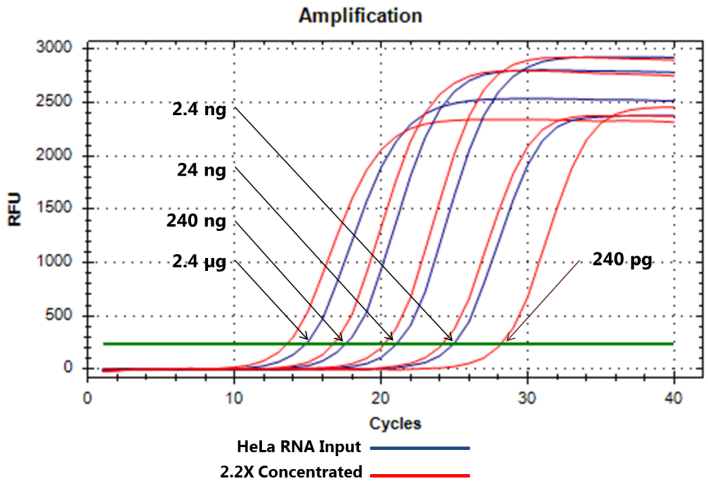

Figure 1. Concentration of Total RNA. The indicated amounts of HeLa RNA were concentrated using the RNA Clean-Up and Concentration Micro-Elute Kit. For each input amount, a 17.5 µL volume was processed and the RNA eluted in 8 µL, resulting in a 2.2 fold concentration. Three microliters of each eluate were used in 10 µL RT reactions with the oligo-dT primer, followed by real-time PCR using 3 µL of the resulting cDNA and primers specific for the human RPS15 gene. On average, amplifications of the concentrated RNA reached threshold 1 Ct value before the input RNA samples, which was expected based on the concentration factor. The lowest input amount used (240 pg) was only amplified when concentrated (most right sample in figure).

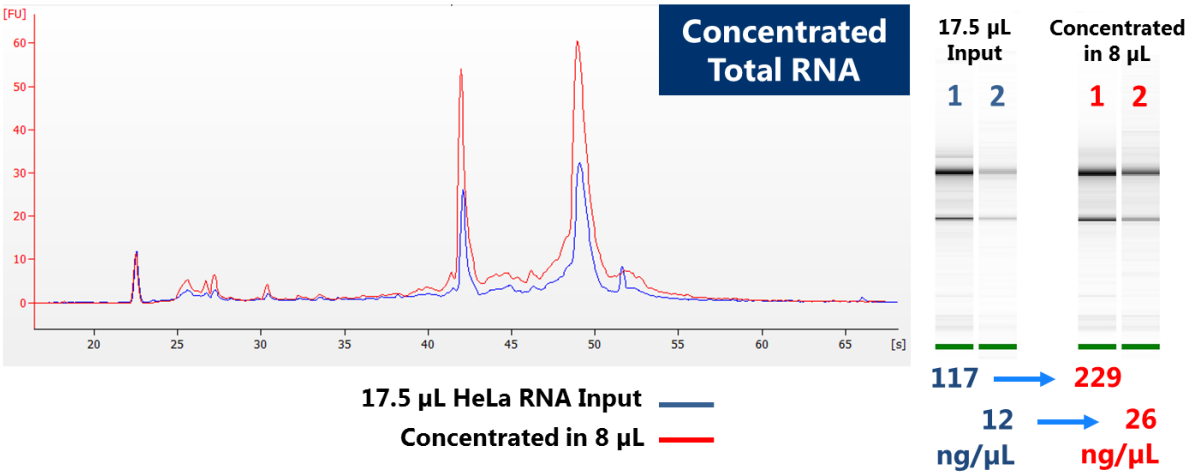

Figure 2. High Recovery of Concentrated RNA. Total RNA isolated from HeLa cells (17.5 µL) was concentrated to 8 µL using the RNA Clean-Up and Concentration Micro-Elute Kit resulting in a 2-2.2 fold increase in RNA concentration, as was measured by Agilent 2100 Bioanalyzer quantification.

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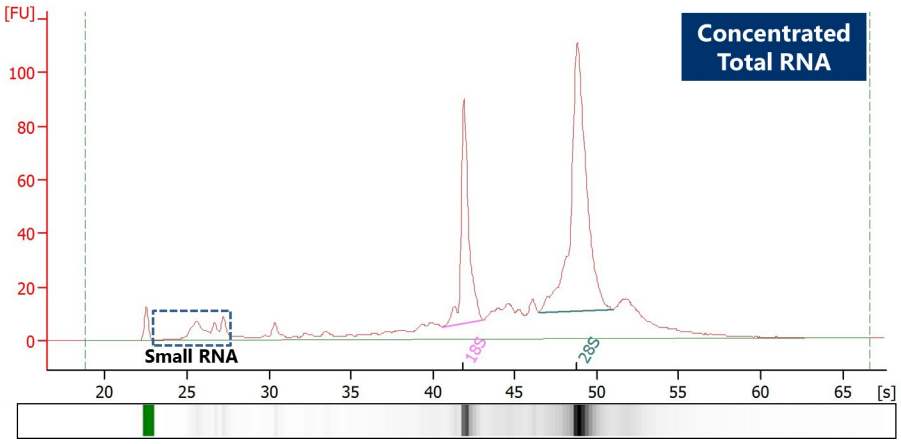
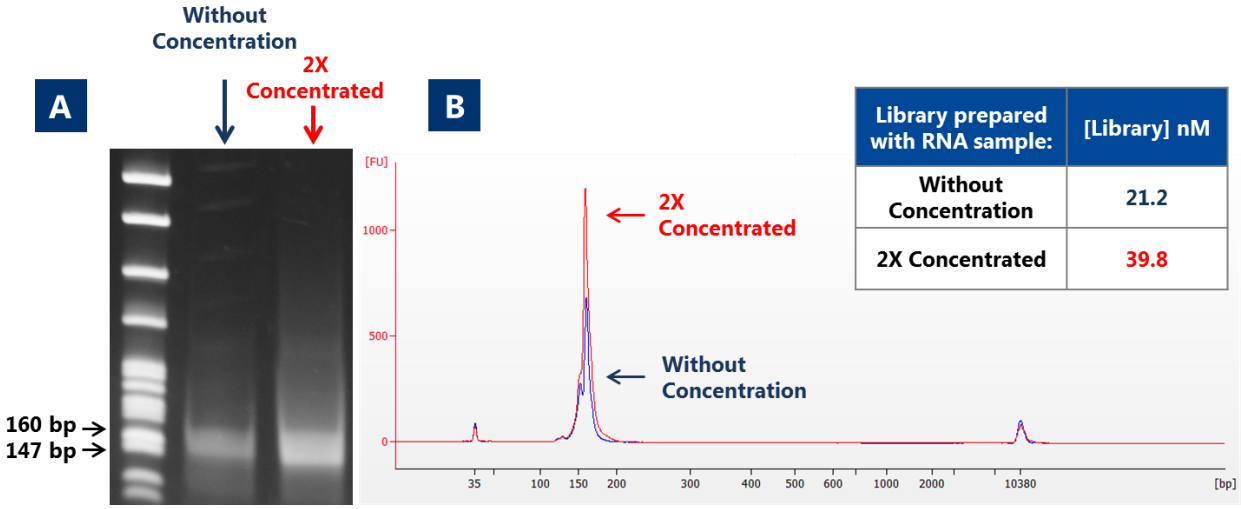


Figure 3. Excellent Quality of Concentrated RNA. Total RNA isolated from HeLa cells (2 µg) was concentrated to 8 µL using the RNA Clean-Up and Concentration Micro-Elute Kit. The excellent quality is indicated by the electropherogram generated using the Agilent 2100 Bioanalyzer (RIN > 9). The concentrated RNA is a true 'total RNA' as can be observed by the presence of small RNA species



Library prepared with RNA sample:	[Library] nM
Without Concentration	21.2
2X Concentrated	39.8

Figure 4. Concentration of RNA prior to Next Generation Sequencing (NGS) applications. Total RNA was purified from 200 µL of plasma collected on EDTA blood tubes using the Total RNA Purification Kit (Cat. 17200) and eluted in 50 µL of elution solution. The same RNA was also concentrated two-fold using the Micro-Elute RNA Column by eluting in 25 µL of elution solution. Five microliters of both the RNA without additional concentration and the 2X concentrated RNA were used as inputs to generate RNA libraries (using the NEBNext® Small RNA Library Prep Set for Illumina® and following manufacturers instructions) for small RNA NGS on the MiSeq (Illumina) platform. A) The prepared small RNA libraries were visualized on a 6% TBE polyacrylamide gel, where the library prepared with 2X concentrated RNA contained more ligated/indexed miRNA cDNA (147-160 bp) products than the library prepared using the RNA without concentration. B) The cDNA was extracted from excised gel bands and interrogated using the Agilent 2100 Bioanalyzer (High Sensitivity DNA Assay). As would be expected based on input, the small RNA library prepared with the 2X concentrated RNA sample was approximately two times more concentrated than the library prepared with RNA without prior concentration (39.8 vs 21.2 nM, respectively).

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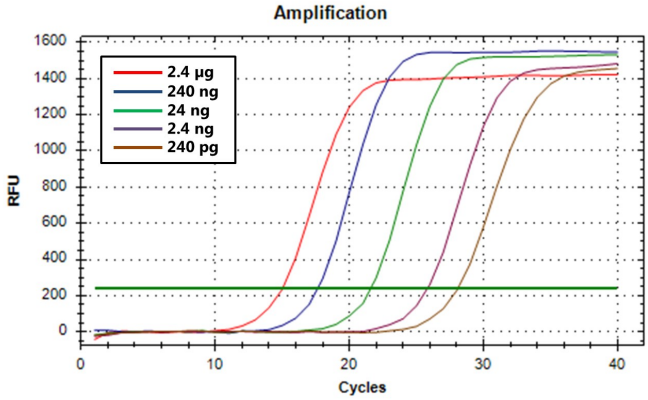

Figure 5. Concentration of miRNA. The indicated amounts of HeLa RNA were concentrated using the RNA Clean-Up and Concentration Micro-Elute Kit. Three microliters of each 8 µL eluate were used in 10 µL RT reactions with the miR-21-SLR primer, followed by real-time PCR using 3 µL of the resulting cDNA and the forward primer specific for miR-21.

Kit Contents:

1. Buffer RL
2. Wash Solution A
3. Elution Solution A
4. Column Activation Solution
5. Micro-Elute RNA Columns
6. Collection Tubes
7. Elution Tubes (1.7 mL)
8. Product Insert

Storage Conditions

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

Customer-Supplied Reagents and Equipment

For RNA Clean-Up and Concentration from Enzymatic Reactions or Previously Isolated RNA

- Benchtop microcentrifuge
- b-mercaptoethanol
- 96 - 100% ethanol
- RNase-free or DEPC-treated water

For RNA Clean-up and Concentration from Aqueous Phase (RNA fraction) of Phenol/Guanidine-Based RNA (Trizol or Tri Reagent) Isolation Methods

- Benchtop microcentrifuge
- 70% ethanol

Shipping Conditions

The RNA Clean-Up and Concentration Micro-Elute Kit is shipped at room temperature.

Cat #	Description	Quantity
61000	RNA Clean-Up and Concentration Micro-Elute Kit	50 preps

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