RNA Clean and Concentrator Kit

Product description

The RNA Clean and Concentrator Kit is designed for rapid and high-throughput purification of RNA obtained from enzymatic reactions such as in vitro transcription. The kit effectively separates RNA from unincorporated NTPs, enzymes, buffers, and other components. It is suitable for quickly removing any large-scale transcription reactions. It can also be used to remove nucleotides, short oligonucleotides, proteins, and salts from RNA.

The process is simple and fast, and effectively recover 1 ng to 500 μg RNA, suitable for purification of ssRNA lager than 100 nt and dsRNA lager than 200 bp. The RNA Clean and Concentrator Kit consists of three steps: RNA binding to the membrane in the filter cartridge, washing away contaminants and elution of RNA in low-salt buffer. The RNA recovered can be used in any application that requires high-purity RNA.

Composition and storage conditions

Components \ Size	20 rxns	Storage
Binding Solution	8 mL	4°C
Wash Solution Concentrate (Add 20 mL 100% ethanol before use)	5 mL	4°C
5 M Ammonium Acetate	1 mL	4°C
Elution Solution	5 mL	4°C
Filter Cartridges & Collection Tubes	20 tubes	Room temperature
Elution Tubes	20 tubes	Room temperature
Shipping: Bluc Ice	**Shelf life:** 12 months	

Experimental Protocol

1. Materials Not Supplied

1) 100% ethanol (ACS grade or better): For preparation of the Wash Solution; For binding RNA to the Filter Cartridge.

2) Equipment to pass solutions through Filter Cartridges:

- Microcentrifuge (required): The microcentrifuge must be capable of attaining 10,000–15,000× g (typically 10,000–14,000 rpm)
- Vacuum manifold (optional): Using a vacuum manifold (with an adequately powerful vacuum pump) is considerably faster than drawing the solutions through the Filter Cartridges with a microcentrifuge. Use 5 mL syringe barrels to support the Filter Cartridges on the vacuum manifold.

2. Experimental preparations

1) Before using the kit for the first time, prepare the Wash Solution:

Add 20 mL of ACS grade 100% ethanol to the bottle labeled Wash Solution Concentrate and mix well. The solution added with ethanol will be called the Wash Solution.

2) Equipment preparation:

Before performing RNA operations, it is best to clean the laboratory bench and pipettors with an RNase decontamination solution. Wear laboratory gloves at any times during this process and change them frequently. Use RNase-free pipette tips to handle the Wash Solution and the Elution Solution, and avoid putting used tips into the reagent containers. Use the Collection and Elution Tubes supplied with the kit.

> ***Note:** Filter Cartridges should not be subjected to RCFs over 16,000× g because it could cause mechanical damage and/or may deposit glass filter fiber in the final sample

3. Procedure

1) Bring the RNA sample to 100 µL with Elution Solution. Mix gently but thoroughly.

2) Add 350 µL of Binding Solution Concentrate to the sample. Mix gently by pipetting.

3) Add 250 µL of 100% ethanol to the sample. Mix gently by pipetting.

4) Apply the RNA mixture sample to the filter:

- Centrifuge users:

a) Insert a Filter Cartridge into 1 of the Collection and Elution Tubes supplied.

b) Transfer the RNA mixture sample into the Filter Cartridge.

c) Centrifuge for ~15 s to 1 min, or until the mixture has passed through the filter. Centrifuge at RCF 10,000–15,000× g (typically 10,000–14,000 rpm). Spinning harder than this may damage the filters.

d) Discard the flow-through and reuse the Collection and Elution Tube for the washing steps.

■ Vacuum manifold users:

a) Put 5 mL syringe barrels on the vacuum manifold, load them with Filter Cartridges, and apply the vacuum.

b) Transfer the RNA mixture sample into the Filter Cartridge. The vacuum will draw it through the filter. Do not worry about the RNA mixture passing through the Filter Cartridges too quickly and preventing RNA from binding, because once the mixture passes through the Filter Cartridges, the RNA will immediately bind.

5) Wash twice with 500 µL Wash Solution:

a) Apply 500 µL Wash Solution. Draw the Wash Solution through the filter as in the previous step 4).

b) Repeat with a second 500 µL aliquot of Wash Solution.

c) After discarding the Wash Solution, continue centrifugation or leave the Filter Cartridge on the vacuum manifold for 10–30 s to remove the last traces of Wash Solution.

*__Note:__ Make sure that the ethanol has been added to the Wash Solution Concentrate before using it.

6) Elute RNA from the filter with 50 µL Elution Solution using one of the methods described below; They are equivalent in terms of RNA recovery:

■ RNA elution method 1

a) Place the Filter Cartridge into a new Collection/Elution Tube.

b) Apply 50 µL of Elution Solution to the center of the Filter Cartridge. Close the cap of the tube and incubate in a heat block set to 65–70°C for 5–10 min.

c) Recover eluted RNA by centrifuging for 1 min at RT (RCF 10,000–15,000×g).

d) To maximize RNA recovery, repeat this elution procedure with a second 50 µL aliquot of Elution Solution. Collect the eluate into the same tube.

■ RNA elution method 2

a) Pre-heat 110 µL of Elution Solution per sample to 95°C.

b) Apply 50 µL of the pre-heated Elution Solution to the center of the Filter Cartridge, close the cap of the tube and centrifuge for 1 min at room temperature (RCF 10,000–15,000 ×g) to elute the RNA.

(c) To maximize RNA recovery, repeat this elution procedure with a second preheated 50 µL aliquot of Elution Solution. Collect the eluate into the same Collection/Elution Tube.

*Note: If glass fibers are observed in your sample, they can be removed by centrifuging the sample briefly and then transferring the RNA to a new tube.

7) Precipitate with 5 M Ammonium Acetate to concentrate the RNA (optional):

a) Add 1:10 volume of 5 M Ammonium Acetate (NH4Ac) to the purified RNA. If the sample is eluted with 100 µL Elution Solution as suggested, this will be 10 µL of 5 M NH4Ac.

b) Add 2.5 times the volumes of 100% ethanol (275 µL if the RNA is eluted in 100 µL). Mix well and incubate at -20°C for 30 min.

c) Microcentrifuge at top speed for 15 min at 4°C or room temperature (RT).

d) Carefully remove and discard the supernatant.

e) Wash with 500 µL70% cold ethanol, centrifuge again and remove the 70% ethanol.

f) To remove the last traces of ethanol, quickly re-spin the tube, and aspirate any residual fluid with a very fine tipped pipette, or with a syringe needle.

g) Air dry the precipitate.

h) Resuspend the precipitate using the desired solution and volume.

4. Assessing RNA yield

Assessing RNA yield by UV absorbance

The concentration of RNA can be determined by diluting an aliquot of the preparation (usually a 1:50 to 1:100 dilution) in TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA), and reading the absorbance in a spectrophotometer at 260 nm. The buffer used for dilution need not be RNase-free (unless you want to recover the RNA), since slight degradation of the RNA will not significantly affect its absorbance.

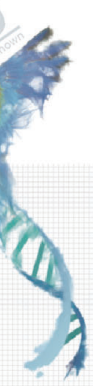
*Note: Be sure to zero the spectrophotometer with the TE buffer used for sample dilution.

An A_{260} of 1 is equivalent to 40 µg RNA/mL. The concentration (µg/mL) of RNA is therefore calculated as follows: A_{260} × dilution factor × 40 µg/mL.

Notes

1. During the experiment, make sure that all reagents and consumables are protected from RNase contamination.

2. This product is for scientific use only!



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