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MEGAclear™ Kit
Purification for Large Scale Transcription Reactions

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Product description

The MEGAclear™ Kit is designed for rapid high-throughput purification of RNA from enzymatic reactions such as in vitro transcription. The process is simple and fast, and it recovers from 1 ng to 500 µg of RNA efficiently. The MEGAclear™ Kit is appropriate for purification of ssRNA larger than 100 nt and dsRNA larger than 200 bp.

The MEGAclear™ Kit procedure consists of three steps:

1. RNA is bound to the membrane in the Filter Cartridge.
2. Contaminants are washed away.
3. RNA is eluted in a low salt buffer.

The MEGAclear™ Kit can be used to remove nucleotides, short oligonucleotides, proteins, and salts from RNA. The RNA recovered can be used for any application that requires high purity RNA. Use the MEGAclear™ Kit to clean up any of the following:

- In vitro transcribed RNA:
  - RNA from MEGAscript® reactions
  - Amino allyl-modified RNA
  - Biotinyled RNA
  - Cy® Dye labeled RNA
  - Capped RNA (e.g. from mMESSAGE mMACHINE® Kit reactions)
- Total RNA

Kit contents and storage

The kit contains reagents for 20 RNA purifications.

Amount	Component	Storage
8 mL	Binding Solution	4°C
5 mL	Wash Solution Concentrate (Add 20 ml 100% ethanol before use)	4°C
1 mL	5 M Ammonium Acetate	4°C
5 mL	Elution Solution	any temperature†
20	Filter Cartridges	room temperature
40	Collection and Elution Tubes	room temperature

† Store at room temperature, 4°C, or –20°C.

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Required materials not provided

100% ethanol: ACS grade or better

- For preparation of the Wash Solution
- For binding RNA to the Filter Cartridge

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.

MEGAclear™ Kit Procedure

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. Mix well. Place a check in the box on the label to indicate that the ethanol was added. With the ethanol, this solution will be referred to as Wash Solution.

Equipment preparation

Lab bench and pipettors

Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g. Ambion® RNaseZap® Solution).

Gloves and RNase-free technique

Wear laboratory gloves at all times during this procedure and change them frequently. They will protect you from the reagents, and they will protect the RNA from nucleases that are present on skin.

Use RNase-free pipette tips to handle the Wash Solution and the Elution Solution, and avoid putting used tips into the reagent containers.

Microfuge tubes

Use the Collection and Elution Tubes supplied with the kit; they have been tested for RNase contamination and are certified RNase-free.

MEGAclear™ Kit Procedure

CAUTION! 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.

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 sample to the filter:
   - **Centrifuge users**:
   a. Insert a Filter Cartridge into 1 of the Collection and Elution Tubes supplied.
   b. Pipet the RNA mixture onto the Filter Cartridge.
   c. Centrifuge for ~15 sec 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. Pipet the RNA mixture onto the Filter Cartridge. The vacuum will draw it through the filter. Do not be concerned if the RNA mixture is pulled through very quickly, the RNA will bind instantly.
5. Wash with 2 × 500 µL Wash Solution.

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

   a. Apply 500 µL Wash Solution. Draw the Wash Solution through the filter as in the previous step.
   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 sec to remove the last traces of Wash Solution.
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 option 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 option 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 x g) to elute the RNA.

c. To maximize RNA recovery, repeat this elution procedure with a second pre-heated 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. (optional) Precipitate with 5 M Ammonium Acetate. To concentrate the RNA, precipitate as follows:
   a. Add 1:10 volume of 5 M Ammonium Acetate (NH_4Ac) to the purified RNA.

   Note: If the sample was eluted with 100 µL Elution Solution as suggested, this will be 10 µL of 5 M NH_4Ac.

   b. Add 2.5 volumes of 100% ethanol (275 µL if the RNA was 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 the pellet with 500 µL 70% 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 pellet.
   h. Resuspend the pellet using the desired solution and volume.

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 (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. Be sure to zero the spectrophotometer with the TE 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.

Assessing RNA yield with RiboGreen® Kit

RiboGreen® Kit provides a sensitive method for quantitating RNA in solution. Follow the manufacturer's instructions for use.

Quality control

Functional testing

An entire MEGAscript® reaction is purified, and recovery is shown to be >75% of input RNA. The RNA is then reverse transcribed using a trace radiolabel and the reaction products are analyzed by PAGE.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

A sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Appendix A Safety

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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The information in this guide is subject to change without notice.

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