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MEGAclear™-96 Kit
High Throughput Purification for Large Scale Transcription Reactions

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Product description

The MEGAclear™-96 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 0.5 to 100 µg of RNA per well efficiently. The MEGAclear™-96 Kit is appropriate for purification of ssRNA larger than 100 nt and dsRNA larger than 200 bp.

The MEGAclear™-96 Kit procedure consists of three steps:

1. RNA is bound to the glass fiber in the Filter Plate.
2. Contaminants are washed away.
3. RNA is eluted in a low salt buffer or in Nuclease-free Water.

The MEGAclear™-96 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™-96 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

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Kit contents and storage

The kit contains reagents for 96 RNA purifications.

Amount	Component	Storage
8 mL	Binding Solution	4°C
20 mL	Wash Solution Concentrate (Add 80 ml 100% ethanol before use)	4°C
10 mL	Elution Solution	any temperature†
20 mL	Nuclease-free Water	any temperature†
1	MEGAclear™-96 Filter Plate	room temperature
1	Collection Plate‡	room temperature
2	Adhesive Plate Sealer	room temperature

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

‡ Collection Plates are not suitable for UV measurements.

Required materials not provided

100% ethanol: ACS grade or better

- For preparation of the Wash Solution
- For binding RNA to the filters in the MEGAclear™-96 Filter Plate

Equipment to pass solutions through Plate Filter Cartridges

- Vacuum pump or in-house vacuum line with at least 5" Hg (630 torr) vacuum
- Vacuum manifold: It is important to apply and maintain vacuum pressure at 5–7" Hg (580–630 torr) for this procedure, therefore we recommend using a vacuum manifold with an integrated vacuum gauge and bleed valve. It is also important to make sure that the manifold is designed to position the filter outlet within 5 mm of the top of the collection plate; if the filter outlet is >5 mm above the collection plate, cross-contamination can occur between adjacent wells.

(Optional) 96-well centrifugation equipment

Centrifuge with rotor and adapters to hold 96 well plate and collection plate assembly capable of ~2,000 x g RCF. The rotors and adapters listed below can be used in Beckman's Allegra 6 or Spinchron DLX centrifuges:

Rotor	Adapter	Maximum RCF
Beckman GH-3.8	MicroPlus Carrier (Beckman #BK362394)	3200 rpm = 1924 × g
Beckman PTS 2000	Microplate Canister (Beckman #BK361301)	3200 rpm = 1924 × g

MEGAclear™-96 Kit procedure

Before using the kit for the first time

Prepare the Wash Solution

Add 80 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.

Collection Plate

Use the Collection Plate supplied with the kit; it has been tested for RNase contamination and is certified RNase-free.

Vacuum manifold set-up

Seat the Filter Plate on the vacuum manifold, and set the bleed gauge so that the vacuum pressure will be 5–7″ Hg (580–630 torr).

MEGAclear™-96 Kit procedure

Note: Set the vacuum pressure to 5–7″ Hg (580-630 torr).

1. If necessary, bring the RNA samples to 20 µL with Nuclease-free Water. Mix gently but thoroughly.
2. Add 70 µL of Binding Solution to the samples. Mix gently by pipetting.
3. Add 50 µL of 100% ethanol to the samples. Mix gently by pipetting.
4. Apply the samples to the filters in the Filter Plate.

   Note: If fewer than 96 wells are used, seal the unused wells with the Adhesive Plate Sealer. Be careful to apply RNA mixtures to the filters at the bottom of the wells of the Filter Plate.

   - **Vacuum manifold users:** Apply vacuum for 2 min. The vacuum will draw the mixtures through the filters. Do not be concerned if the RNA mixtures are pulled through very quickly, the RNA will bind instantly.
   - **Centrifuge users**: Make sure to use a receptacle with enough capacity to catch the flow-through. Centrifuge at 1,900 × g for 3 min.

5. Wash with 2 × 300 µL Wash Solution.

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

   a. Apply 300 µL Wash Solution. Draw the Wash Solution through the filter as in the previous step.

   b. Repeat with a second 300 µL aliquot of Wash Solution.

   c. **Centrifuge users only**: After discarding the Wash Solution, continue centrifugation for 10–30 sec to remove the last traces of Wash Solution.

6. (optional) **Vacuum manifold users only**: Wash with 200 µl 100% Ethanol.

   This step quickly dries the filter to ensure that no ethanol will be carried over into the eluted RNA; it is not necessary for centrifuge users.

   Apply 200 µl 100% Ethanol and apply vacuum for 3 min.

7. Elute RNA with 2 × 50 µL Elution Solution or Nuclease-free Water.

   IMPORTANT! Elution Solution contains 0.1 mM EDTA; if this amount of EDTA could interfere with your downstream application, use the Nuclease-free Water supplied with the kit instead.

   IMPORTANT! Use gentle vacuum pressure (5–7" Hg or 580–630 torr) for the elution. Higher force may blow the eluted RNA out of the Collection Plate.

   a. Pre-heat 10 mL Elution Solution (or Nuclease-free Water) per plate to 95° C.

   b. Place the Collection Plate underneath the Filter Plate. Apply 50 µL of the pre-heated Elution Solution (or Nuclease-free Water) to the center of each filter in the Filter Plate, apply vacuum gently for 3 min or centrifuge at 1,900 x g for 3 min to elute the RNA.

   c. To maximize RNA recovery, repeat this elution procedure with a second pre-heated 50 µL aliquot of Elution Solution (or Nuclease-free Water). Collect the eluate into the same Collection Plate.

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 1:25 to 1:50 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

A 200 µL MEGAscript® reaction is split into 10 samples and purified in 10 random wells on a single MEGAclear™-96 Filter Plate. Recovery is shown to be >75% of input RNA with <10% well-to-well deviation.

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

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