DNAclear™ Kit

Purification and Concentration of Double-stranded cDNA
Part Number AM1756

Applied Biosystems

A. Product Description

The DNAclear™ Purification Kit is designed to purify double-stranded DNA from enzymatic reactions such as second strand cDNA synthesis and PCR. The buffers and protocol are adapted from the purification method used in the Ambion® MessageAmp™ aRNA Amplification Kits. The process is simple and fast, and it efficiently recovers and concentrates from 1 ng to 3 µg of DNA per purification. The DNAclear procedure consists of three steps; (1) DNA is mixed with cDNA Binding Buffer and bound to the filter in the Micro Filter Cartridge, (2) contaminants are washed away, and (3) the DNA is eluted in water. The DNAclear Kit can be used to remove nucleotides, short oligonucleotides, proteins, and salts from DNA. The DNA recovered can be used for any application that requires high purity DNA, such as in vitro transcription.

B. Reagents Provided with the Kit

The kit contains reagents for 20 DNA purifications.

Amount	Component	Storage
5.6 mL	cDNA Binding Buffer	room temp*
12 mL	cDNA Wash Buffer (add 9.6 mL 100% ethanol before use)	room temp
20	Micro Filter Cartridges + Collection Tubes	room temp
20	Micro Elution Tubes	room temp
5 mL	Nuclease-free Water	any temp†

* The cDNA Binding Buffer may form a precipitate if stored below room temp. If a precipitate is visible, redissolve it by warming the solution to 37°C for up to 10 min and vortexing vigorously. Cool to room temp before use.
† Store Nuclease-free Water at –20°C, 4°C or room temp

C. Required Materials Not Provided

- 100% ethanol: ACS grade or better, for the preparation of cDNA Wash Buffer
- Microcentrifuge capable of RCF 10,000 X g

D. DNAclear Procedure

IMPORTANT

The DNA binding capacity of the Micro Filter Cartridges is 1 ng to 3 µg. If purifying a PCR product we recommend applying a maximum of 50 µL of a reaction. Applying more than 3 µg will result in loss of DNA.

IMPORTANT

Micro Filter Cartridges should not be subjected to RCFs over 16,000 X g because it could cause mechanical damage and/or may deposit glass filter fiber in the eluate. All centrifugations in this procedure should be done at 10,000 X g (typically ~10,000 rpm) at room temperature.

Before using the kit for the first time, prepare the cDNA Wash Buffer

- Add 9.6 mL ACS grade 100% ethanol to the cDNA Wash Buffer. Mix well.
- Check the box on the label to indicate that the ethanol was added.

1. Preheat Nuclease-free Water to 55°C

Before beginning the procedure, preheat the 5 mL bottle of Nuclease-free Water to 55°C for at least 10 min.

2. Equilibrate one Micro Filter Cartridge per sample

Immediately before starting the cDNA purification, equilibrate one Micro Filter Cartridge for each sample:

a. Add 30 µL cDNA Binding Buffer to a Micro Filter Cartridge assembled in a Collection Tube.

b. Incubate 5 min at room temp. (The cDNA Binding Buffer does ***not*** need to be spun through.)

3. Bring sample volume to 100 µL with Nuclease-free Water

If the DNA sample volume is less than 100 µL, bring it to 100 µL using Nuclease-free Water.

IMPORTANT

Check the cDNA Binding Buffer for precipitation before using it, and if a precipitate is visible, redissolve it by warming the solution to 37°C for up to 10 min and vortexing vigorously. Cool to room temp before use.

4. Add 250 µL cDNA Binding Buffer

Add 250 µL of cDNA Binding Buffer to each sample, and mix thoroughly by repeated pipetting or gentle vortexing.

5. Apply the mixture to an equilibrated Micro Filter Cartridge

a. Pipet the mixture from step 4 into an equilibrated Micro Filter Cartridge (from step 2).

b. Centrifuge for ~1 min at 10,000 x g, or until the mixture is through the filter.

c. Discard the flow-through and replace the Micro Filter Cartridge in the Collection Tube.

6. Wash the Micro Filter Cartridge with 500 µL cDNA Wash Buffer

Make sure that the ethanol has been added to the bottle of cDNA Wash Buffer before using it.

a. Apply 500 µL cDNA Wash Buffer to each Micro Filter Cartridge.

b. Centrifuge for ~1 min at 10,000 x g, or until all the cDNA Wash Buffer is through the filter.

c. Discard the flow-through and spin the Micro Filter Cartridge for an additional minute to remove trace amounts of ethanol.

d. Transfer the Micro Filter Cartridge to a Micro Elution Tube, and discard the Collection Tube.

7. Elute cDNA with 2 aliquots of preheated Nuclease-free Water

The elution volume is flexible; for maximum DNA concentration elute with 16 µL (2 x 8 µL), or for more efficient DNA recovery elute with up to 100 µL (2 x 50 µL).

a. Apply 8–50 µL Nuclease-free Water (preheated to 55°C) to the filter in the Micro Filter Cartridge.

b. Leave at room temperature for 2 min and then centrifuge for ~1 min at 10,000 x g, or until all the Nuclease-free Water is through the filter.

c. Repeat the elution with a second 8–50 µL of preheated Nuclease-free Water. The double-stranded cDNA will now be in the Nuclease-free Water in the Micro Elution Tube.

d. Discard the Micro Filter Cartridge.

8. (optional) Check the dsDNA on an agarose gel

If ≥1 µg of total RNA or ≥30 ng of poly(A) RNA was used for aRNA amplification, there may be enough dsDNA at this point in the procedure to visualize it on an agarose gel. Run 1 µL of the sample on a 1% agarose TBE gel using ethidium bromide (or another nucleic acid dye) to visualize the DNA.

E. Assessing DNA Yield by UV Absorbance

The concentration of DNA 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. Be sure to zero the spectrophotometer with the TE used for sample dilution.

An A_{260} of 1 is equivalent to 50 µg DNA/mL.

Following is a typical example:

DNA is eluted in 100 µL

5 µL of the prep is diluted into 395 µL (1:80) of TE

A_{260} = 0.34

DNA conc. = 0.34 x 80 x 50 µg/mL = 1360 µg/mL or 1.36 µg/µL

There are 95 µL remaining after using 5 µL to measure the concentration, the total amount of remaining DNA is:

95 µL x 1.36 µg/µL = 129.2 µg

F. Quality Control

Functional Testing

The kit is tested for percent DNA recovery using a PCR product. Recovery of differently sized DNAs are tested using a DNA marker.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

G. Safety Information

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs; previously known as MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety goggles, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Obtaining the MSDS

To obtain Safety Data Sheets (SDSs) for any chemical product supplied by Applied Biosystems or Ambion are available 24 hours a day. At www.appliedbiosystems.com, select Support, then SDS/MSDS. Search by chemical name, product name, product part number, or SDS/MSDS part number. Right-click to print or download the SDS of interest. At www.ambion.com, go to the web catalog page for the product of interest. Select SDS/MSDS, then right-click to print or download. Or e-mail (MSDS_Inquiry_CCRM@lifetech.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated SDSs unless you request fax or postal delivery. Requests for postal delivery require 1-2 weeks for processing

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