

Section E

# Nucleic Acid Research Kits

Kits for cDNA synthesis,  
amplification, and normalization



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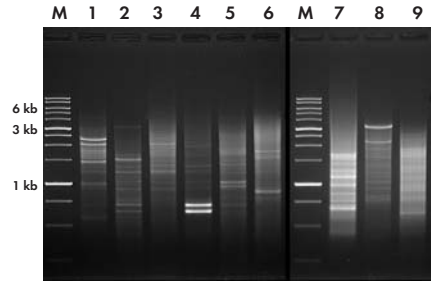
## MINT cDNA synthesis kits

- Fast cDNA synthesis protocol
- High content of full length transcripts
- Low background
- Small starting amount of poly(A+) or total RNA required

### Product description

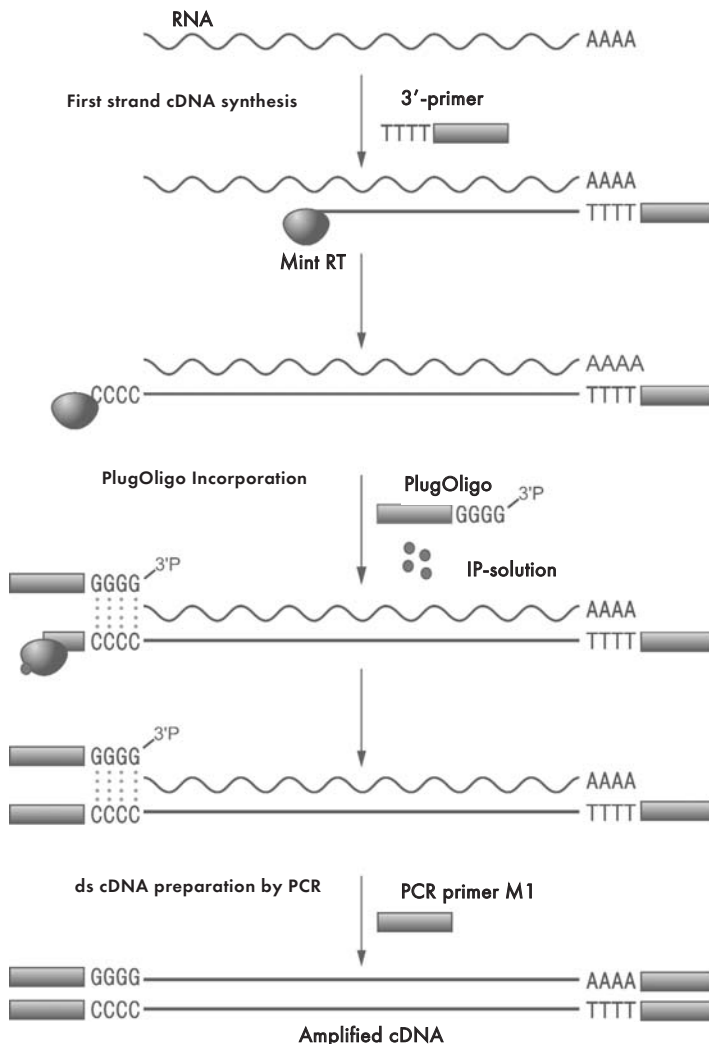
Evrogen offers cDNA synthesis kits designed to generate full-length-enriched double stranded (ds) cDNA from a total or poly(A+) RNA. Depending on your particular need, you can select Mint-Universal or Mint kit, where the first allows preparation of cDNA ready for either nondirectional or directional cloning and the second — for nondirectional cloning only.

Synthesized cDNA can be used in various applications including cDNA library construction; Virtual Northern blot; suppression subtractive hybridization (SSH); cDNA normalization using Trimmer or Trimmer-Direct



**Mint-amplified cDNA from different sources.**

1 — Mouse liver; 2 — mouse skeletal muscle; 3 — mouse brain; 4 — human leucocytes; 5 — human lung; 6 — human skeletal muscle; 7 — mosquito grub; 8 — copepod *Pontella* sp.; 9 — tomato *Lycopersicon esculentum*. M — 1 kb DNA size markers, SibEnzyme, Russia.



### Schematic outline of Mint cDNA synthesis.

Mint cDNA synthesis is based on a novel technology (patent pending) using the specific features of Mint reverse transcriptase (RT).

First strand cDNA synthesis starts from the 3'-end adapter comprising oligo(dT) sequence that anneal to poly(A+) stretch of RNA. When RT reaches the 5'-end of the mRNA, it adds several non-template nucleotides, primarily deoxycytidines, to the 3'-end of the newly synthesized first-strand cDNA [1]. This oligo(dC) stretch base pairs to complementary oligo(dG) sequence located at the 3'-end of a special 30-mer deoxyribooligonucleotide called PlugOligo. RT identifies PlugOligo as an extra part of the RNA-template and continues synthesis of the first strand cDNA to the end of the oligonucleotide, thus incorporating PlugOligo sequence into the 5'-end of cDNA.

The last 3'-dG residue of the PlugOligo is a terminator nucleotide comprising 3'-phosphate group. This blocking group prevents unwanted annealing and extension of the PlugOligo. Under standard conditions, RT can hardly use PlugOligo as a template, however, our special IP-solution (solution for Incorporation of PlugOligo sequence) dramatically increases the efficiency of this process.

At the last step, ds cDNA is amplified by PCR. Use of Encyclo polymerase and specially designed primers allows synthesis of full-length-enriched cDNA that is flanked by PlugOligo and 3'-end adapter sequences.

Product	Cat.#	Size
Mint cDNA synthesis kit	SK001	20 rxn
Mint-Universal cDNA synthesis kit	SK002	20 rxn

The kits are not available in certain countries. Please contact your local distributor or Evrogen before ordering.

Kit components must be stored at -20°C.

### Important note for kit selection

Adapters used to synthesize cDNA for the subsequent direct cloning of the library are longer than those used to prepare cDNA for nondirectional cloning. Use of the longer adapters leads to a reasonable decrease in the cDNA average length and often to the appearance of a low-molecular-weight fraction in the cDNA (which in turn makes it necessary to include a size-separation procedure to remove short cDNA fragments before cloning). Therefore, if directional cloning of cDNA library is not critical to your research, we recommend that you use the Mint kit (Cat.# SK001) or Mint-Universal kit Protocol-I.

**Each kit comprises** a detailed instruction and reagents for 20 cDNA synthesis reactions including a free Mint reverse transcriptase sample and a trial-size Encyclo PCR kit allowing accurate cDNA amplification.

Mint kit comprises adapter pair for synthesis of cDNA flanking with symmetric sequences. Mint-Universal kit comprises two pairs of adapters for synthesis of cDNA flanking with symmetric or asymmetric sequences.

Mint-Universal kit does not include columns for size-fractionating of cDNA, both do not include materials for cDNA purification. These materials must be purchased separately.

### Related services

- cDNA preparation — see page F-4
- cDNA normalization — see page F-6
- cDNA depletion — see page F-8
- cDNA subtraction — see page F-9

### References

1. Schmidt and Mueller (1999) *Nucleic Acids Res.* 27(21): e31.

### Notice to Purchaser:

The products are covered by Evrogen Patents and/or Patent applications pending. By use of this product, you accept the terms and conditions of the applicable Limited Use Label License (see Appendix B, page G-6).

PCR process is subject to patents issued in certain countries. Some elements of this material may be also covered by third party patents issued and applicable in certain countries. No license under these patents is conveyed expressly or by implication to the recipient of the material. Users of this material may be required to obtain a patent license depending upon the particular application and country in which the material is received or used.

### MATERIAL SAFETY DATA SHEET INFORMATION

To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.

## Encyclo PCR amplification kit

- High yield of PCR products from a wide variety of templates
- Suitable for difficult templates
- PCR up to 15 kb

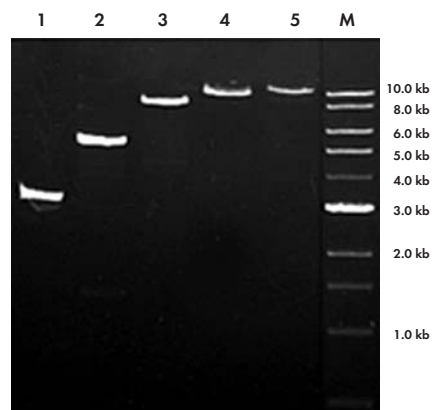
### Product description

Encyclo PCR kit is intended for most PCR applications. It is especially recommended for cDNA amplification because of optimal combination of high fidelity and processivity provided by Encyclo polymerase mix.

Evrogen Encyclo polymerase mix produces high yields of PCR products from a wide variety of templates and displays following features:

- 5'>3' DNA polymerase activity with high processivity
- Proofreading 3'>5' exonuclease activity
- Automatic hot start
- TA cloning compatibility

Encyclo buffer has been developed to facilitate the amplification of specific PCR products and to provide successful amplification of long DNA templates. Encyclo PCR kit includes a mix of high-purity deoxyribonucleotides as well as sterile PCR water. Control DNA template and primer mix enclosed can be used for positive control PCR reaction.



**PCR amplification of phage lambda DNA fragments using "Encyclo PCR kit".**

1 – 2.9 kb; 2 – 5.0 kb; 3 – 8.0 kb; 4 – 10.0 kb;  
5 – 15.0 kb. M – 50 ng of DNA size markers  
(1 kb DNA ladder, NEB).

Product	Cat.#	Size
Encyclo PCR kit	PK001	100 rxn

The kit is not available in certain countries. Please contact your local distributor or Evrogen before ordering

Kit components must be stored at -20°C. Kit includes components for 100 PCR reactions of 50 µl each.

### Notice to Purchaser:

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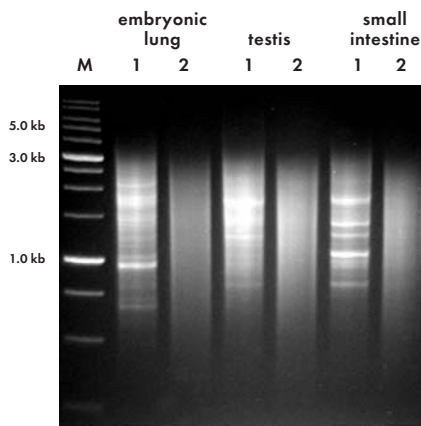
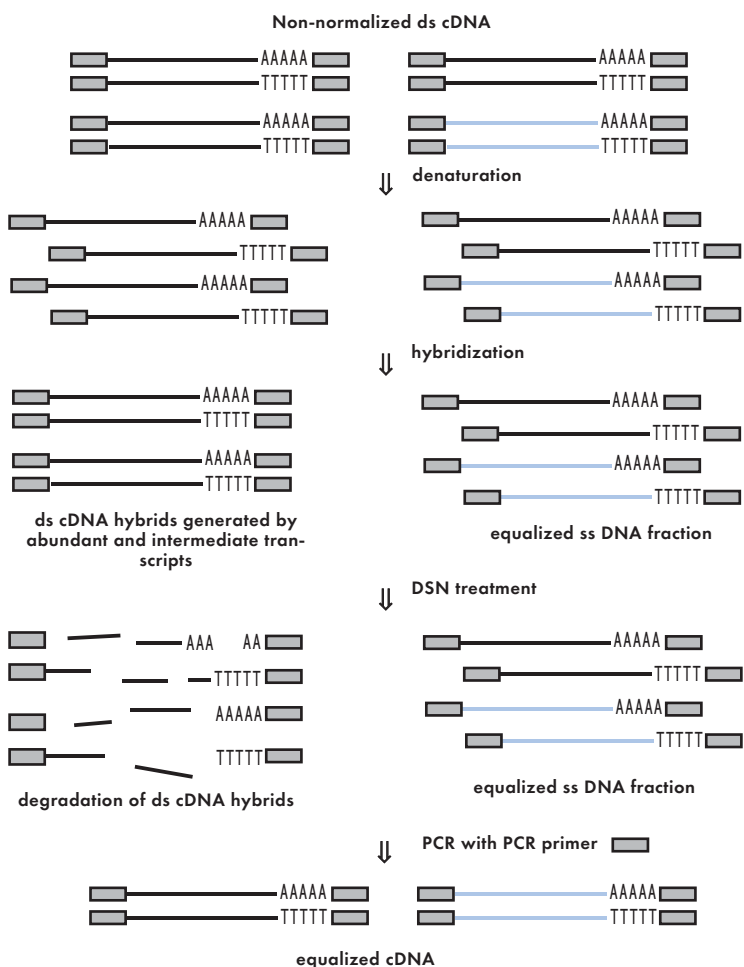
# TRIMMER cDNA normalization kits

- Acceleration of EST projects, transcriptome analysis, functional screening, and rare gene discovery
- Rapid and reliable way to remove repeated transcripts from cDNA library
- Equalization of full-length-enriched cDNA before library cloning
- Simple procedure, no physical separation steps

## Product description

Evrogen offers cDNA normalization kits designed to generate full-length-enriched double stranded (ds) cDNA with equalized concentrations of different transcripts. Both total RNA and poly(A+) RNA can be used for cDNA preparation and subsequent normalization.

Depending on your particular needs, you can select Trimmer or Trimmer-Direct kit wherein the first allows preparation of normalized cDNA ready for nondirectional cloning and the second — for directional cloning.



### cDNA normalization result.

Agarose gel electrophoresis of non-normalized (lanes 1) and Trimmer-Direct-normalized (lanes 2) amplified SMART-prepared cDNA from different human tissues. M — 1 kb DNA size markers, SibEnzyme, Russia.

### DSN normalization scheme.

Black lines represent abundant transcripts, blue lines — rare transcripts. Rectangle represents adapter sequence and its complement. Evrogen normalization kits are based on a DSN-normalization technology [1,2]. The method involves denaturation-reassociation of cDNA, degradation of dsDNA fraction formed by cDNA encoding abundant transcripts and PCR amplification of the equalized ssDNA fraction. The key element of this method is degradation of dsDNA fraction using Kamchatka crab duplex-specific nuclease (DSN). DSN is a thermostable enzyme specific to the dsDNA [3]. Normalization is done before cDNA cloning, and it does not include physical separation steps. Because of specific cDNA synthesis procedure, 5'- and 3'-adapters comprise common external sequence that is used for PCR amplification of normalized cDNA fraction. A specific, suppression PCR-based approach, prevents reduction of the average cDNA length during PCR [4].

Product	Cat.#	Size
Trimmer cDNA normalization kit	NK001	10 rxn
Trimmer -Direct cDNA normalization kit	NK002	10 rxn

Please contact your local distributor for exact prices and delivery information.

Each kit comprises a detailed instruction and reagents for 10 cDNA normalization reactions. Kits do not include materials for cDNA preparation, amplification, and size-fractionation. These materials must be purchased separately. Please use the following instruction (section "Starting materials") to select kits suitable for cDNA synthesis.

Kit components must be stored at -20°C. Lyophilized DSN enzyme must be stored at +4°C before reconstitution.

### Starting materials

For cDNA normalization using Trimmer kit, cDNA must be prepared using one of the following cDNA synthesis kits:

- Mint cDNA synthesis kit (Evrogen Cat.# SK001);
- Mint-Universal cDNA synthesis kit, Protocol-I (Evrogen Cat.# SK002);
- SMART™ PCR cDNA synthesis kit (Clontech Cat.# 634902).

For cDNA normalization using Trimmer-Direct kit, cDNA must be prepared using one of the following cDNA synthesis kits:

- Mint-Universal cDNA synthesis kit, Protocol-II (Evrogen Cat.# SK002);
- SMART™ cDNA Library Construction kit (Clontech Cat.# 634901)\*;
- Creator™ SMART™ cDNA Library Construction kit (Clontech Cat.# 634903)\*.

\*Please follow instruction provided in Trimmer-Direct kit for cDNA synthesis using these kits. Please note that CDS-3M adapter (provided in Trimmer-Direct kit) must be used for cDNA preparation instead of CDS primer included into Clontech kits.

### Important note for kit selection

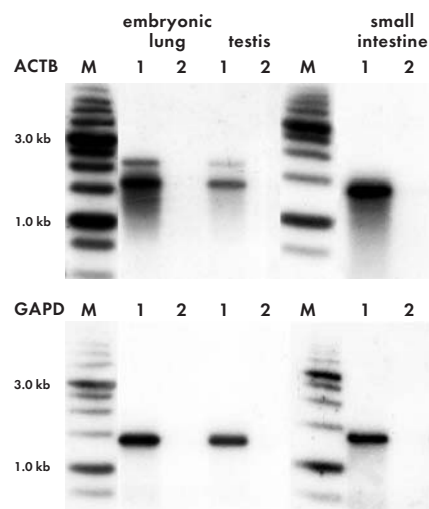
Trimmer and Trimmer-Direct kits are based on the similar technology, but utilize cDNA synthesized using different adapters. Adapters used to synthesize cDNA for the subsequent directional cloning of the library are longer than those used to prepare cDNA for nondirectional cloning. Longer adapters leads to a reasonable decrease in the cDNA average length and often to the appearance of a low-molecular-weight fraction in the cDNA (which in turn makes it necessary to include a size-separation procedure to remove short cDNA fragments before cloning). Therefore, if directional cloning of cDNA library is not critical to your research, we recommend that you use the Trimmer kit (Cat.# NK001).

### Notice to Purchaser:

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### MATERIAL SAFETY DATA SHEET INFORMATION

To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.



### Virtual Northern blot analysis of abundant transcripts in the cDNA samples from human tissues.

1 — Non-normalized cDNA; 2 — Trimmer-Direct-normalized cDNA; ACTB — beta-actin; GAPD — glyceraldehyde-3-phosphate dehydrogenase.

M — 1 kb DNA size markers, SibEnzyme, Russia.

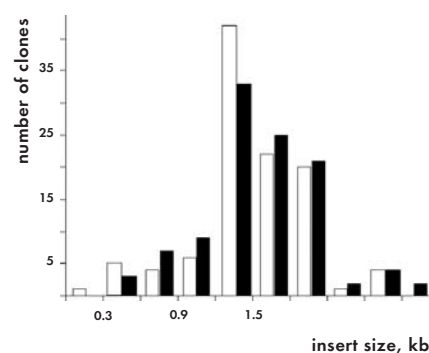


Diagram of the insert size distribution in the non-normalized (white columns) and Trimmer-normalized (black columns) human skeletal muscle cDNA libraries.

### Related services

- cDNA normalization — see page F-6

### References

1. Zhulidov *et al.* (2004) *Nucleic Acid Res.* 32: e37.
2. Zhulidov *et al.* (2005) *Russian Journal of Bioorganic Chemistry* 31 (2):170-177.
3. Shagin *et al.* (2002) *Genome Res.* 12, 1935-1942.
4. Shagin *et al.* (1999) *Nucleic Acids Res.* 27(18): e23.

## Duplex-specific nuclease

- Nuclease specific to double-stranded DNA
- Thermostable
- Inhibited by EDTA
- Recommended for cDNA normalization, cDNA depletion, and for other application to hydrolyze dsDNA in a complex nucleic acid samples

### Product description

Duplex-specific nuclease (DSN) is an enzyme purified from hepatopancreas of the Kamchatka crab [1]. DSN shows a strong preference for cleaving double-stranded DNA and DNA in DNA-RNA hybrid duplexes, compared with single-stranded DNA and RNA. Moreover, the cleavage rate of short, perfectly matched DNA duplexes by this enzyme is considerably higher than that for nonperfectly matched duplexes of the same length.

DSN acquires its enzymatic activity in the presence of  $Mg^{2+}$  ions (at least 5 mM is required for most applications) and is inhibited by EDTA. The pH and temperature optima for activity are 7-8 and 55-65°C, respectively. The nuclease is stable at a wide range of pH (from 4 to 12) and temperatures below 60°C. Moreover, 60% of DSN activity remains after incubation at 70°C for 30 min, and 40% — after incubation at 80°C. In addition, DSN is tolerant to proteinase K treatment (for 30 min at 37°C).

Product	Cat.#	Size
Duplex-specific nuclease	EA001	50 Units
Duplex-specific nuclease	EA002	100 Units
Duplex-specific nuclease	EA003	10 Units

Please contact your local distributor for exact prices and delivery information.

Each package comprises a detailed instruction, lyophilized DSN, and DSN storage and working buffers. Lyophilized DSN enzyme must be stored at +4°C before resolution. Other components should be stored at -20°C.

DNAase activity was measured using modified Kunitz assay, where unit definition was defined as: the amount of DSN added to 50 µg/ml calf thymus DNA that causes an increase of 0.001 absorbance units per minute. Activity assay was performed at 25°C, in 50 mM Tris-HCl buffer, pH 7.15, containing 5 mM  $MgCl_2$ .

### References

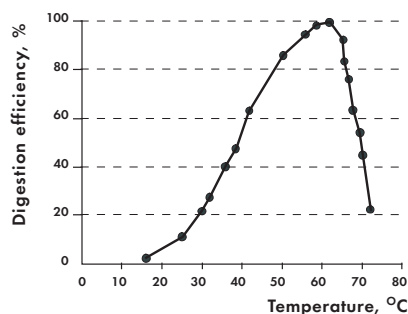
1. Shagin *et al.* (2002) *Genome Res.* 12, 1935-1942.

### Notice to Purchaser:

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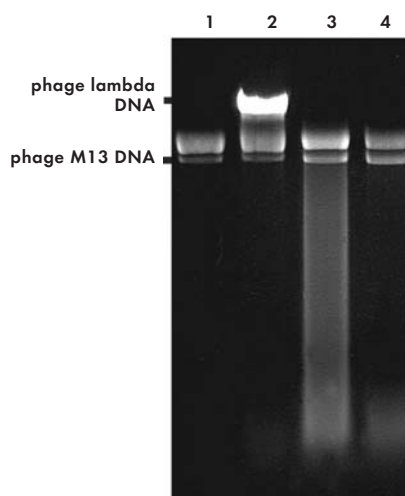
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### Dependence of the DSN activity from temperature.

Activity of DNAse on ds DNA substrate was measured using Kunitz assay at different temperature.



### Action of DSN on ss DNA of phage M13 and ds DNA of phage lambda.

Lanes 1, 2 — negative controls, incubation without nuclease. 1 — phage M13 DNA alone, 2 — mixture containing phage M13 and lambda DNA. Lanes 3; 4 — digestion of phage M13 and lambda DNA mixture by DSN at 70°C for 1.5 min (lane 3) and 5 min (lane 4).