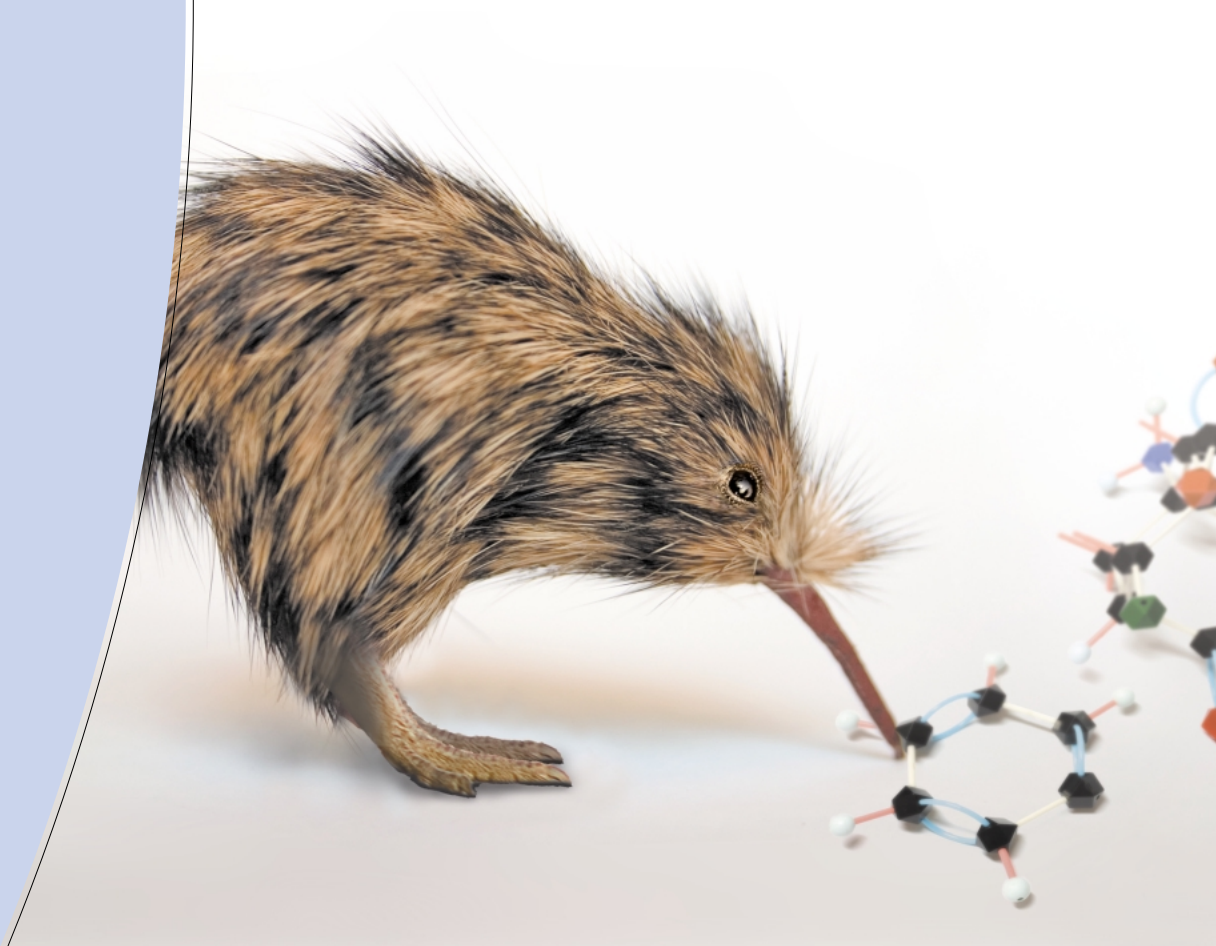
Nucleic Acid Research Kits

- Kits for cDNA synthesis, amplification, and normalization



Contents

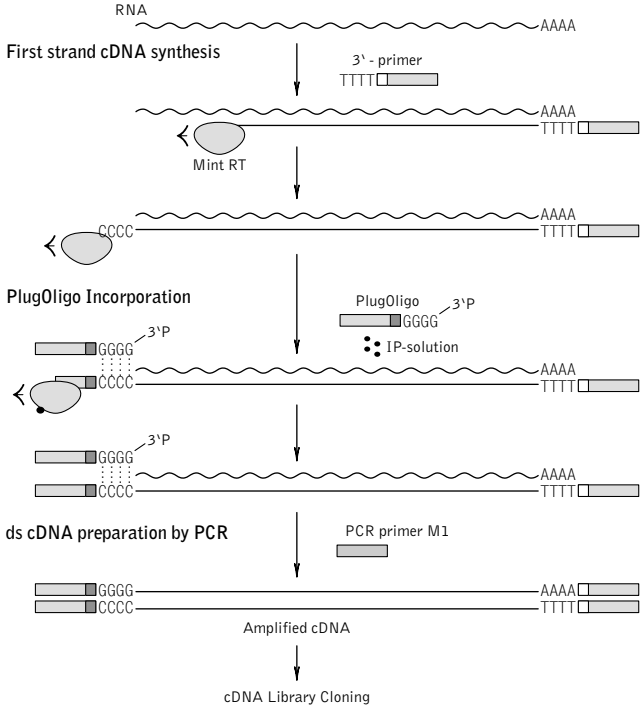
Mint cDNA synthesis kits

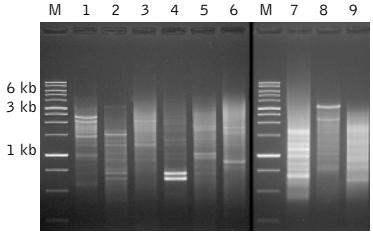
- Fast cDNA synthesis protocol
- High content of full length transcripts
- Low background
- Small starting amount of poly(A)+ or total RNA required
- Compatibility with Trimmer cDNA normalization kits

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.

Evrogen offers cDNA synthesis kits designed to generate full-length-enriched double stranded (ds) cDNA from 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 allows for nondirectional cloning only.





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. First strand cDNA synthesis starts from the 3`-end oligo(dT) primer 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 [Schmidt and Mueller 1999]. This oligo(dC) stretch base pairs to complementary oligo(dG) sequence located at the 3`- end of a special 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 hardly uses 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.

Each kit is comprised of 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 contains 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.

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 kits (Cat. NK001; NK002, please see page 112 for description).

REFERENCES

Schmidt and Mueller (1999). Nucleic Acids Res, 27 (21): e31 / pmid: 10518626

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	Cat.#	Size
Encyclo PCR kit	PK001	100 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.

Product description

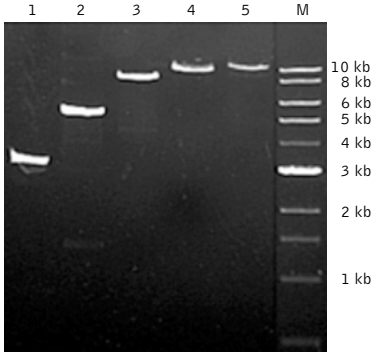
Encyclo PCR kit is suitable for most PCR applications. It is especially recommended for cDNA amplification because of optimal combination of high fidelity and processivity provided by Encyclo polymerase mix.

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.

Kit includes components for 100 PCR reactions of 50 μl each.



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).

TRIMMER
cDNA normalization kits

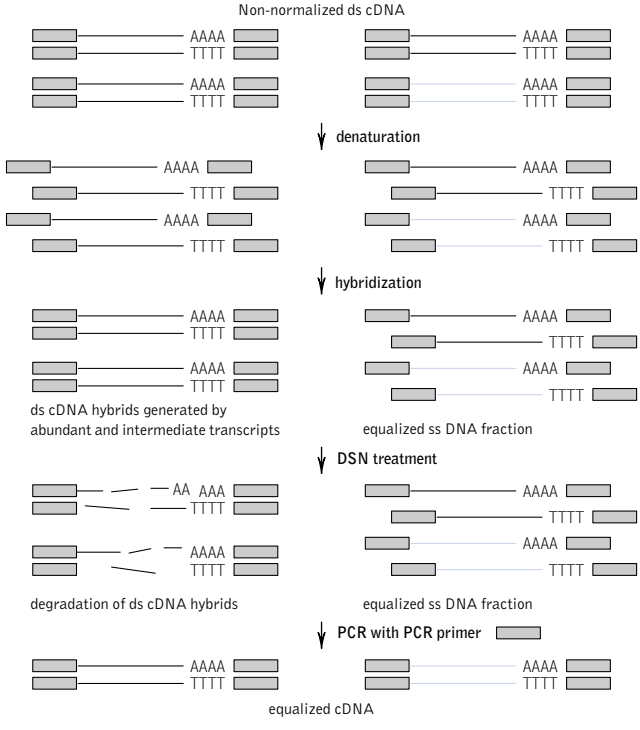
- Rapid and reliable way to remove repeated transcripts from cDNA library
- Equalization of full-length-enriched cDNA before library cloning
- Simple procedure, no physical separation steps
- Recommended for EST projects, transcriptome analysis, functional screening, and rare gene discovery

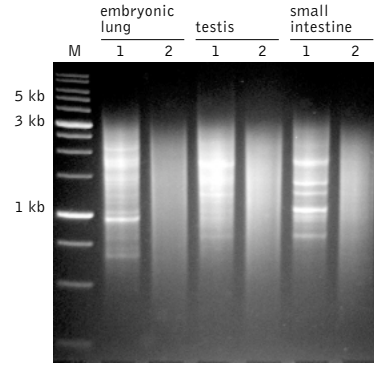
Product	Cat.#	Size
Trimmer cDNA normalization kit	NK001	10 rxn
Trimmer-Direct cDNA normalization kit	NK002	10 rxn

Please contact your local distributor for exact prices and delivery information. Kit components must be stored at -20°C. Lyophilized DSN enzyme must be stored at +4°C before reconstitution.

Evrogen offers cDNA normalization kits designed to generate full-length-enriched double stranded (ds) cDNA with equalized concentrations of different transcripts.

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 allows 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 [Zhulidov et al. 2004, [Zhulidov et al. 2005]. 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 termostable enzyme specific to the dsDNA [Shagin et al. 2002]. Normalization is done before cDNA cloning, and it does not include physical separation steps. Because of specific cDNA synthesis procedure, 5`n- 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 [Shagin et al. 1999].

Each kit contain 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.

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, see page 109);
- Mint-Universal cDNA synthesis kit, Protocol-I (Evrogen Cat.# SK002, see page 109);
- SMART™ PCR cDNA synthesis kit (Clontech Cat.# 634902).

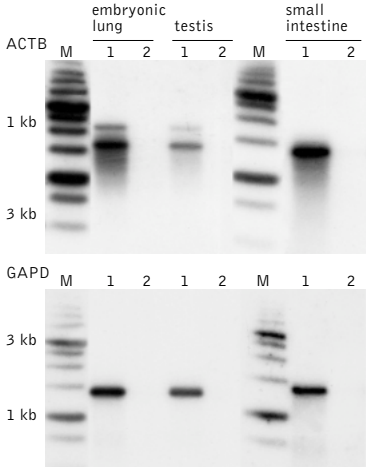
For cDNA normalization using Trimmer-Direct kit, cDNA must be prepared using one of the following cDNA synthesis kits:

- Mint-Universal cDNA synthesis kit, Protocol-II (Evrogen Cat.# SK002, see page 109);
- 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 similar technology, but utilize cDNA synthesized employing 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).



Virtual Northern blot analysis of abundant transcripts in the cDNA samples from human tissues.
1 - Non-normalized cDNA; 2 - Trimmer-Direct-normalized cDNA; ACTB - β-actin; GAPD - glyceraldehyde-3-phosphate dehydrogenase.
M - 1 kb DNA size markers, SibEnzyme, Russia.

REFERENCES

Shagin et al. (1999). Nucleic Acids Res, 27 (18): e23 / pmid: 10471753

Shagin et al. (2002). Genome Res, 12 (12): 1935–1942 / pmid: 12466298

Zhulidov et al. (2004). Nucleic Acids Res, 32 (3): e37 / pmid: 14973331

Zhulidov et al. (2005). Bioorg Khim., 31 (2): 186–194 / pmid: 15889793

Duplex-specific nuclease

- Nuclease specific to double-stranded DNA
- Thermostable
- Inhibited by EDTA
- Recommended for cDNA normalization, cDNA depletion, and for other applications requiring selective removal of dsDNA from complex nucleic acid samples

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

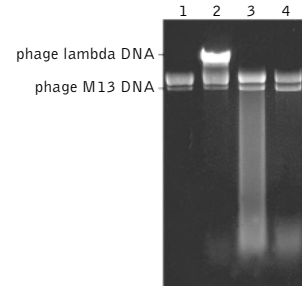
Please contact your local distributor for exact prices and delivery information. Kit components must be stored at -20°C. Lyophilized DSN enzyme must be stored at +4°C before reconstitution.

Duplex-specific nuclease (DSN) is an enzyme purified from hepatopancreas of the Kamchatka crab [Shagin et al. 2002]. 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.

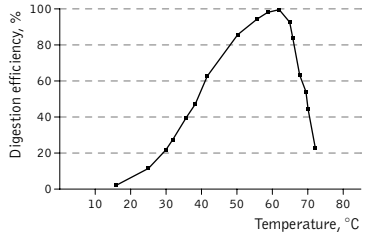
DSN acquires its enzymatic activity in the presence of Mg^{2+} ions (at least 5 mM is required for most applications) and is inhibited by EDTA. The pH and temperature optima for activity are 7-8 and 55-65°C, respectively. The nuclease is stable at a wide range of pH (from 4 to 12) and temperatures below 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).

Each package comprises a detailed instruction, lyophilized DSN, and DSN storage and working buffers.

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 $MgCl_2$.



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 (3) and 5 min (4).



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

REFERENCES

Shagin et al. (2002). Genome Res, 12 (12): 1935–1942 / pmid: 12466298