



IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt

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PCRTerminator[®] End Repair Kit

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PCRTerminator[®] End Repair Kit

Components & Storage Conditions

The PCR Terminator End Repair Kit contains one tube each of End Repair Enzyme Mix and 5X End Repair Buffer in the following amounts:

PCRTerminator Kit Components			Store at -20°C
Catalog #	Reactions	End Repair Enzyme	5X End Repair Buffer
40037-1	10	25 µl	100 µl
40037-2	20	125 µl	500 µl

Description

Amplification by the polymerase chain reaction (PCR) requires the use of one of several thermal stable DNA polymerases. These DNA polymerases can be categorized as either proofreading polymerases (those with an inherent 3' exonuclease activity) or non-proofreading polymerases (those without 3' exonuclease activity). Amplification products generated using proofreading polymerases such as Vent[®] or Pfu are primarily blunt-ended. These products can be cloned without end repair, provided phosphate groups are added to the 5' termini. Amplification products generated with non-proofreading polymerases such as *Taq*, *Tth*, or *Tfl* primarily have single-base adenine extensions at their 3' termini. Consequently, these products must either be inserted into a vector that contains complimentary 3' thymidine extensions or be treated with the PCRTerminator Kit to remove the adenine extensions. They also need to contain 5' phosphate groups to allow cloning into dephosphorylated vectors.

The PCRTerminator Kit contains a mixture of enzyme activities that has been optimized to create blunt, 5'-phosphorylated ends on any type of PCR product. After treatment with the PCRTerminator Kit, insertion of PCR products into blunt vectors, such as the high efficiency pSMART[®] vectors, can be several orders of magnitude more efficient than T/A cloning.

Materials and Equipment Needed

The PCRTerminator End Repair Kit has been optimized for processing one to five µg of PCR product. Common molecular biology equipment, supplies, and reagents are required.

Buffers used for PCR must be removed before beginning the PCRTerminator reaction. We recommend electrophoresis on an agarose gel, followed by purification of the desired band using a commercial purification column (e.g., Zymoclean Gel DNA Recovery Kit, Zymo Research, cat. # D4001; www.zymoresearch.com). Gel fractionation assures cloning of the correct PCR product rather than truncated variants, aberrant products, or multimers of PCR primers.

If the DNA is not gel fractionated, it can be purified using commercial purification kits (e.g., DNA Clean & Concentrate Kit, Zymo Research, cat. # D4003) or ethanol precipitation. The DNA should be eluted or dissolved in water.

Following the PCRTerminator End Repair reaction, the DNA must be purified again using commercial purification kits or ethanol precipitation to remove the repair enzymes.

PCRTerminator[®] End Repair Kit

PCRTerminator End Repair Reaction

Mix the following components in a microfuge tube:

37.5 μ l purified amplification product (1-5 μ g)
10.0 μ l 5X PCRTerminator End Repair Buffer
1.0 - 2.5 μ l PCRTerminator End Repair Enzyme*
<hr/>
50.0 μ l final volume

- * Use 2.5 μ l End Repair enzyme to treat > 2 μ g amplification product.
- Use 1 μ l of End Repair enzyme to treat 1-2 μ g amplification product.
- For less than 1 μ g of amplification product, reduce incubation time to 15 minutes.

Incubate 30 minutes at room temperature.
Stop the reaction by incubation at 70°C for 15 minutes.
Purify the DNA as described above.

Note: the heat denaturation step may be omitted if the DNA is *immediately* purified using a protein denaturing reagent (e.g., phenol or column binding buffer that contains guanidinium HCl).

Tips on Maximizing Yield of Transformants

Avoid exposing the DNA to short wave UV radiation

If the PCR product is visualized by UV after gel purification, use of a hand-held long wavelength (360 nm) lamp is highly recommended. Using a short wavelength (254 or 302 nm) ultraviolet transilluminator to visualize DNA in the gel can reduce cloning efficiencies by several orders of magnitude.

Do not exceed the recommended incubation time for end repair

Exceeding the 30-minute incubation time can lead to nucleolytic degradation of the fragments, which can reduce the number of transformants. For less than 1 μ g of product, reduce the amount of PCRTerminator End Repair Enzyme to 1.0 μ l, and reduce the incubation time to 15 minutes.

Purify the PCR products before and after the end repair step

Purification maximizes the efficiency of end repair and subsequent cloning reactions.

Quantify the DNA before and after the end repair reaction

The PCRTerminator Kit is designed to treat one to five micrograms of PCR product. Using an amount of input DNA outside this range can alter the kit's effectiveness. Care must be taken to accurately quantitate the insert DNA before and after end repair. We recommend that a portion of the end-repaired sample be quantitated by gel electrophoresis with mass standards.

Avoid using ammonium ions prior to end repair

Ammonium ions interfere with the end repair reactions. Avoid introducing ammonium to the fragments prior to the end repair step. (i.e. do not precipitate with ammonium acetate prior to the end repair step).