BIO BASIC
Quality-Affordable Research

Product information

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dT-Tailing Kit

Catalog #: BS71217
Size: 100 preps
Storage: -20°C*

*: Product will be shipped with ice pack. Check storage conditions.

Product Description:

This kit is designed to add a single deoxythymidine (dT) residue at the 3' end of any blunt-end DNA fragments (normally a vector) based on the non-template-dependent terminal transferase activity of Taq polymerase. This T residue on the vector will hybridize to the single A overhang on the Polymerase Chain Reaction (PCR) product and increase the efficiency of ligation.

Storage:

Transportation on ice pack. Store at -20°C for 12 months.

Composition:

2 × dT-Tailing Buffer	2 x 1.25 ml
Taq DNA Polymerase (5 U/µl)	2 x 50 µl
Protocol	1

Procedures:

1. Blunt-end DNA fragments preparation

Blunt-end DNA fragments can be generated by digestion of plasmid (such as pUC19) with a blunt-end restriction enzyme (such as EcoR V) or by PCR using a DNA polymerase with proofreading activity (such as pfu). DNA should be purified by gel extraction, phenol:chloroform method or using a DNA clean up kit (Cat#BS367).

2. dT-Tailing reaction

In a thin-walled tube, add the following components:

Purified DNA fragments	0.2-2 µg
2 × dT-Tailing Buffer	25 µl
Taq DNA Polymerase (5 U/µl)	1 µl
ddH_2O	up to 50 µl

Mix thoroughly and incubate at 72°C for 2 hours.

3. Post Treatment

The dT-tailed DNA should be purified before further application, phenol:chloroform method is recommended.

a) Add 50 µl of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) to the reaction mixture and mix well.

b) Centrifuge in a microcentrifuge at 12,000 rpm for 10 minutes to separate the phases.

c) Transfer the aqueous phase (upper phase) to a fresh microcentrifuge tube.

d) Add 50 µl of Chloroform and mix well.

e) Centrifuge at 12,000 rpm for 10 minutes to separate the phases.

f) Transfer the aqueous phase (upper phase) to a fresh microcentrifuge tube.

g) Add 100 µl of ice-cold ethanol and mix well.

h) Centrifuge at 12,000 rpm for 10 minutes to pellet the DNA.

i) Discard the supernatant, add 100 µl of ice-cold 70% ethanol and mix well. Centrifuge at 12,000 rpm to pellet the DNA.

j) Repeat this step once.

k) Discard the supernatant and air dry the DNA pellet.

l) Dissolve the DNA pellet in ddH_2O or TE buffer.

m) For ligation reactions, use approximately 25 to 50 ng of T-Vector.