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DNA dA-Tailing Kit

Store at -20°C

Cat. No.	Description	Quantity
E009	DNA dA-Tailing Kit	25 rxns

Product Description
The DNA dA-Tailing Kit efficiently adds a non-template dAMP (dA) to the 3' end of a blunt-ended DNA fragment. This incorporated 3'-dA prevents concatemer formation and prepares the DNA fragment for subsequent ligation of adaptors or cloning vectors that have complementary 3'-dT overhangs.

The Klenow Fragment (3´→ 5´ Exo-) provided in this kit adds the 3'-dA via its DNA polymerase activity. This enzyme lacks 5'→3' exonuclease activity and has mutations that effectively abolish the inherent 3'→5' exonuclease activity, thus preventing degradation of this 3'-dA. The DNA dA-Tailing Kit has been optimized to prepare compatible overhangs for the next step of DNA sample preparation for next generation sequencing. The Klenow Fragment (3´→ 5´ Exo-) is also available as a stand-alone enzyme (Cat. No. E038).

Kit Components

Part No.	Product Components	Volume
E009-1	Klenow Fragment (3'→5' Exo-) (5 U/µl)	100 µl
E009-2	10X dA-Tailing Reaction Buffer	200 µl

Product Applications
- DNA sample preparation
- dA-Tailing of 1-5 µg of blunt ended DNA

Storage Conditions
Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

Enzyme Storage Buffer
25 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, and 50% (v/v) Glycerol.

Enzyme Unit Definition:
One unit is defined as the amount of Klenow Fragment (3'→5' Exo-) that catalyzes the incorporation of 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C using poly(dA-dT):poly(dA-dT) as a template:primer.

Protocol

1. Add the following components to a sterile tube sitting on ice:

Component	Volume	Final Concentration
End repaired, blunt-ended DNA (100-1000 bp)	Variable	1 – 5 µg/rxn
Klenow Fragment (3'→5' Exo-) (5 U/µl)	3 µl	15 U
10X dA-Tailing Reaction Buffer	5 µl	1X
Nuclease-free H_2O	up to 50 µl	-

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 30 minutes.

3. Purify the DNA using column purification (Column-Pure PCR Clean-up Kit, Cat# D509).

4. The 3'dA-tailed DNA product is ready for immediate downstream applications or for long-term storage at -20°C.