M5 Klenow 片段（3'→5' exo-）使用说明书

产品名称	单位	货号
M5 Klenow 片段（3'→5' exo-）	200u	MF463-01
M5 Klenow 片段（3'→5' exo-）	1000u	MF463-05

【储存条件】: -20℃

【产品简介】

Klenow Fragment, exo-, is the large fragment of DNA polymerase I . It exhibits 5'→3' polymerase activity, but lacks the 3'→5' and 5'→3' exonuclease activities of DNA Polymerase I. The 3'→5' exonuclease activity of the enzyme is eliminated by mutations in the 3'→5'-exonuclease active site.

- Lacks 3'→5' exonuclease activity
- Incorporates modified nucleotides (e.g., Cy3-, Cy5-, fluorescein-, rhodamine-, aminoallyl-, biotin-labeled nucleotides)
- Active in restriction enzyme, PCR, and RT buffers

【产品应用】

- Random-primed DNA labeling
- Labeling by fill-in 5'-overhangs of dsDNA
- Strand displacement amplification (SDA)
- DNA sequencing by the Sanger method

【产品组成】

M5 Klenow 片段（3'→5' exo-）(5U/ul)	200U	1000U
10X Klenow Buffer	0.5ml	2x1.5ml

【产品来源】

An E. coli strain containing a plasmid with a fragment of the E. coli polA (D355A, E357A) gene starting at codon 324.

【活性定义】:

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

【分子量】: 68kDa

【热失活】: 75℃ 20 分钟。

【酶切反应条件】:

1X Buffer. Supplement with dNTPs (not included).
Klenow Fragment (3´→ 5´ exo－) is also active in all other Buffers when supplemented with dNTPs.

【缓冲液】:
50 mM NaCl，10 mM Tris-HCl，10 mM MgCl2，1 mM DTT，pH 7.9 @ 25°C

【操作步骤】:

A-Tailing with Klenow Fragment (3'-->5' exo-)

Starting Material:

1-5 µg of blunt-ended DNA* (100-1000 bp).

*If starting with blunt-ended DNA that has been prepared by PCR or by end polishing, DNA must be purified to remove the blunting enzymes.

1. Mix the following components in a sterile microfuge tube:

Purified Blunt DNA:	1-5 µg
10X Klenow Buffer:	5 µl
dATP (10 mM):	0.5 µl (0.1 mM final)
Klenow Fragment (3´→ 5´ exo－):	3 µl
Sterile H_2O:	variable
Total volume:	50 µl

2. Incubate in a thermal cycler for 30 minutes at 37°C.

3. Purify DNA sample on one spin column.