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Instruction manual Blunting high 0810

F0990K

Blunting high

BLK-101 20 reactions
Store at -20 °C.

Contents

CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precautions and follow safety guidelines while using this kit.

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[1] Introduction

Description

Blunting high is a kit that produces a blunt end at the DNA terminus, and allows for its use in a subsequent ligation step using KOD DNA polymerase[1)] and DNA ligase reagents, respectively.

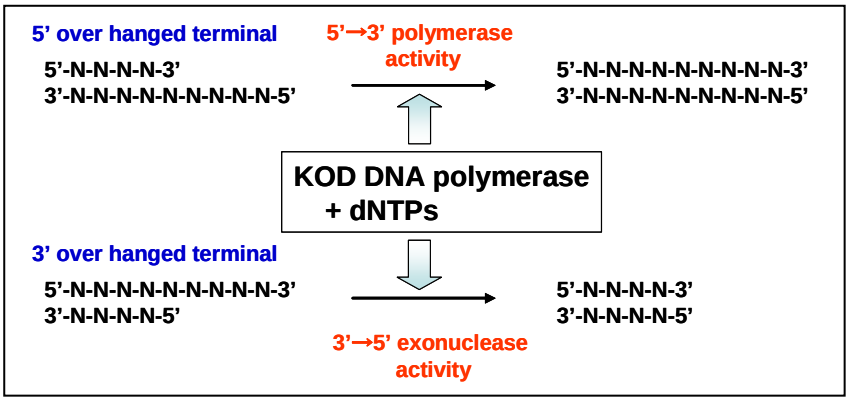

Fig. 1. Principle of Blunting by KOD DNA polymerase

Features

- The blunting step makes use of KOD DNA polymerase that exhibits 5'→ 3' polymerase and 3'→5' exonuclease activities.

- The blunting step is completed in 2 min.

- A highly efficient premixed ligation reagent, "Ligation high" is included in this kit.

- A control reaction can be performed using the control DNA provided.

[2] Components

This kit includes the following components for 20 reactions. All reagents should be stored at - 20 °C.

KOD DNA Polymerase [KOD] (2.5 U/µl)	20 µl
10 x Blunting Buffer	100 µl
Ligation high	375 µl
Control DNA (10 ng/ml)	50 µl

Notes:

- Ligation high is a highly efficient premixed ligation reagent

- The control DNA is pUC18 plasmid digested by *Eco*RI and *Sph* I. Following blunting and subsequent ligation, the circular control plasmid DNA generates *E. coli* colonies on an ampicillin plate.

- 10 x Blunting Buffer contains 1.2 M Tris-HCl (pH 8.0 at 25 °C), 100 mM KCl, 15 mM $MgCl_2$, 60 mM $(NH_4)_2SO_4$, 2 mM dNTPs, 1% TritonX-100, and 0.01% BSA.

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[3] Protocol

1. Blunting

(1) Prepare the following reagents.

Distilled water	8-X µl
DNA solution	X µl
10 x Blunting Buffer	1 µl
KOD (2.5 U/µl)*	1 µl
Total Volume	10 µl

Control reaction

Distilled water	3 µl
Control DNA	5 µl
10 x Blunting Buffer	1 µl
KOD (2.5 U/µl)*	1 µl
Total Volume	10 µl

* KOD (2.5 U/µl) should be added last. This enzyme exhibits strong 3' →5' exonuclease activity in the absence of dNTPs.

(2) Incubate at 72 °C for 2 min.

(3) Place on ice. → [3] 2. Ligation

Notes

Sample DNA

For this kit, DNA should be dissolved in distilled water or TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). However, in the following cases, DNA solutions may be used undiluted, directly with this kit.

- Restriction enzyme-treated DNA

Restriction enzyme-treated solutions using High, Medium, Low or Tris-Acetate buffer can be directly used according to the above protocol.

- PCR products of Taq DNA polymerase

Perform the following protocol.

PCR solution (amplified by Taq-based polymerase)
↓← equal units of KOD (2.5 U/µl)*
72 °C for 2 min

*10 x Blunting Buffer should not be added.
*If the concentration of dNTPs in the PCR solution is less than 0.2 mM, add dNTPs up to 0.2 mM.

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DNA amount

This kit can process 10 pmol blunt DNA per reaction. 10 µg pUC18 (linearized; 2.7 kb) corresponds to 10 pmol. The maximum volume of DNA solution is 8 µl.

Reaction

When preparing the reaction solution, KOD (2.5 U/µl) should be added last. KOD DNA polymerase exhibits strong 3' →5' exonuclease activity in the absence of dNTPs.

Reaction time

The reaction is completed within 2 min. The reaction time can be prolonged up to 30 min.

2. Ligation

(1) Prepare the following reagents.

TE buffer or Distilled water	10-(X+Y) µl
Blunted DNA (from [3] 1. Blunting)	X µl
Vector DNA	Y µl
Ligation high	10 µl
Total Volume	20 µl

Control reaction

TE buffer or Distilled water	8 µl
Blunted Control DNA (from [3] 1. Blunting)* or not blunted Control DNA	2 µl
Ligation high	10 µl
Total Volume	20 µl

*5 ng/µl

(2) Incubate at 16 °C for 1 h

Notes

- The reacted solution can be directly used for transfection experiments using *E. coli*-competent cells.
- For the control experiment, *E. coli* colonies can be observed on ampicillin plates. The colony number in the control experiment using the blunted control DNA will be at least 20 times greater than that using the not blunt control DNA.
- KOD DNA polymerase should not be inactivated prior to the ligation step because this polymerase is hardly active at 16 °C.
- The reacted solution from the blunting reaction can be used directly in a ligation reaction, but excessive carry-over of the solution reduces ligation efficiency. In such cases, prolong the reaction time up to overnight, or purify the blunted products prior to ligation.

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[4] Troubleshooting

Symptom	Cause	Solution
Low efficiency	Addition of too much reacted mixture from the blunting reaction to the ligation reaction	Addition of too much reacted mixture from the blunting reaction to the ligation reaction tends to decrease the ligation efficiency. Decrease the volume of the reacted mixture from the blunting reaction or exchange the buffer of the reacted mixture obtained from the blunting reaction to a low salt solution (e.g. distilled water or TE buffer) with ethanol precipitation etc.
	DNA is degraded	KOD DNA polymerase degrades DNA from the 3' end by its 3'→5' exonuclease activity in the absence of dNTPs. KOD DNA polymerase must be added last.

[5] References

1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.*, 63: 4504-10 (1997)

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