Instruction manual A-attachment mix 0810

F1018K

10 x A-attachment mix

TAK-301 25 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

10 x A-attachment mix is a reagent comprising anti-KOD DNA polymerase antibody specific to KOD 3'→5' exonuclease activity (proof-reading activity) [1], as well as Taq DNA polymerase, which exhibits terminal transferase activity. PCR products from KOD-Plus- [Code No. KOD-201] and KOD FX [Code No. KFX-101] possess blunt ends due to 3'→ 5' exonuclease activity of the KOD DNA polymerase. The 10 x A-attachment mix allows for PCR products to acquire overhanging dA at the 3'-ends. Products with 3'-dA overhangs can be directly cloned into arbitrary T-vectors using ligation reagents, such as Ligation high Ver.2 [Code No. LGK-201].

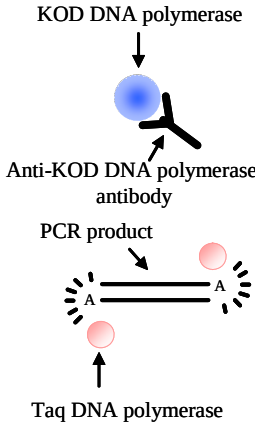

Fig. 1. Principle of the 10 x A-attachment mix

Features

- The attachment reaction is completed in 10 minutes at 60°C.

- The cloning efficiency of dA-attached PCR products is as great as PCR products from Taq DNA polymerase.

[2] Components

This reagent includes the following components for 25 reactions. All reagents should be stored at - 20°C.

10 x A-attachment mix	25 µl

Notes:

-This reagent does not contain buffer components, dNTPs, or magnesium, *etc*. When using purified PCR products and prior to adding 10 x A-attachment mix, PCR buffer, dNTPs, and (magnesium*) must be added to the purified DNA solution to create a 1x PCR reaction solution.

*If magnesium is contained in the PCR buffer, the addition of magnesium is not necessary.

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[3] Protocol

The A-attachment reaction should be performed just prior to the ligation reaction

1. Transfer 9 µl of PCR products* to a fresh tube.

*PCR products from KOD-Plus- [Code No. KOD-201] or KOD FX [Code No. KFX-101] should be used without purification.

Notes

10 x A-attachment mix does not contain buffer components, dNTPs, or magnesium, etc. When using purified PCR products and prior to adding 10x A-attachment mix, PCR buffer, dNTPs, and (magnesium*) must be added to the purified DNA solution to create a 1x PCR reaction solution.

*If magnesium is contained in the PCR buffer, the addition of magnesium is not necessary.

2. Add 1 µl of 10 x A-attachment mix and mix well.

3. Incubate at 60°C for 10 minutes.

Notes

This reaction can be prolonged up to 30 minutes. In most cases, efficiency can be reached in 10 minutes, but maximum efficiency is achieved with 30 minutes.

4. The reaction mixture* can be used directly in the ligation reaction** with T-vector.

*The reaction mixture should be stored at 2-10°C until further use.
**A highly efficient ligation reagent, such as Ligation high Ver.2 [Code No. LGK-201], should be used.

A typical ligation reaction condition is as follows:

Distilled water	X µl
T vector (50 ng/µl)	1-2 µl
dA-attached PCR products*	1-2 µl
Ligation high Ver.2 [Code No. LGK-201]	7.5 µl
Total Volume	15 µl

→ 16°C, 30 minutes

*Too many products will decrease the efficiency

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[4] Examples

Example 1. TA cloning of a 500 bp-PCR product amplified with KOD-Plus-

One microliter of 10 x A-attachment mix was added to 9 µl of unpurified 500 bp-PCR product amplified by KOD-Plus- [Code No. KOD-201]. The reaction was incubated at 60°C for 10 minutes. Subsequently, a solution (7.5 µl) containing 1.5 µl treated PCR products and 75 ng T-vector was mixed with 7.5 µl Ligation high Ver.2 [Code No. TAK-301] and incubated at 16°C for 30 minutes. *E. coli* DH5α competent cells were then transformed using 10 µl of the ligation mixture, and were cultured on LB/Amp (X-gal) plates overnight at 37°C. Blue and white colonies were quantified, and the insert DNA was confirmed by colony-directed PCR using eight white colonies as templates.

As shown in Fig. 1, cloning efficiency was increased by treating PCR products with 10 x A-attachment mix. All eight white colonies were determined to possess the 500-bp inserts (data not shown).

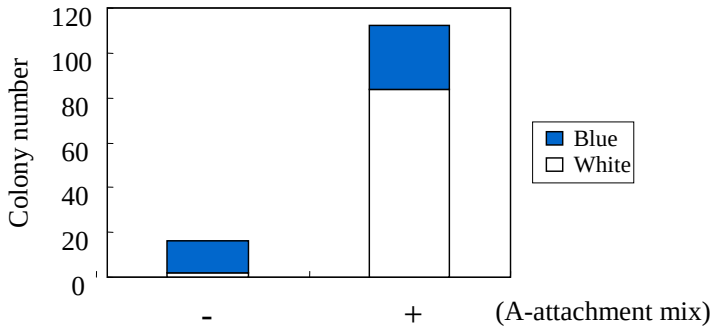

Fig. 1 Comparison of TA cloning efficiency of PCR products from KOD-Plus-.

[5] Reference

1) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem*., 126: 762-8 (1999)

[6] Related products

Product name	Package	Code No.
Highly efficiency ligation solution **Ligation high Ver.2**	750 µl	LGK-201

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