AccuRapid™ TA Cloning Kit

[Cat. No.] K-7170

Introduction

AccuRapid™ TA Cloning Kit is designed for directional cloning of PCR products amplified with *Taq* DNA polymerase into linearized T-vectors without any restriction sites or modifications. *Taq* DNA polymerase adds a single nucleotide, deoxyadenosine (dA) to the 3'-end of the amplified PCR products. The linearized T-vector has a single deoxythymidine (dT) at the 3'-end. The TA Cloning allows the hybridization of the complementary 3'-end A overhangs of PCR products and 3'-end T overhangs of linearized T-vector.

Features & Benefits

- Convenient: Easy excision of DNA insert using *Bam*H I and M13 primer sites for sequencing.
- Rapid: 15 min reaction, enables cloning of various sizes of insert DNA.
- Blue/white screening: Provides blue/white selection function through *LacZα* gene.

Components

Components	Amount
pBHA-T vector (25 ng/μl)	40 μl
Control Insert (70 ng/μl)	20 μl
AccuRapid™ 2X Reaction buffer	100 μl
T4 DNA Ligase (200 U/μl)	20 μl

*** Note**: For research use only. Not for use in diagnostic or therapeutic procedures.

Storage

- Store at -20°C.

Online Resources

Korean

English

Visit our **product page** for additional information and protocols.

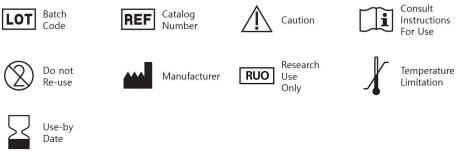
Ordering Information

Description	Cat. No.
AccuRapid™ TA Cloning Kit	K-7170

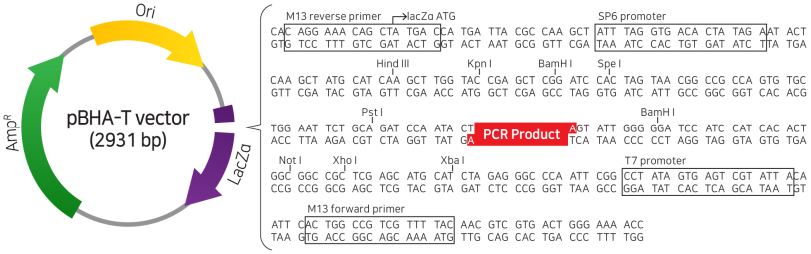
Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



pBHA-T Vector Map



pBHA-T Vector: 2,931 bp

M13 Reverse primer site: bases 1-17

LacZα gene: bases 13-441

SP6 promoter: bases 35-53

T7 promoter: bases 236-254

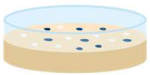
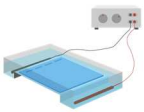
M13 Forward primer site: bases 261-277

Ampicillin resistance gene: bases 1,003-1,863

pUC *ori* (high copy number): bases 2,034-2,622

Copyright 2022 BIONEER Corporation. All Rights Reserved.

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Preparation of PCR products</td><td>1. Perform PCR with Taq DNA polymerase.
* Note: For efficient A-tailing of PCR products, it is recommended to proceed with final extension at 72°C for more than 5 min.
2. Confirm the amplified PCR products by agarose gel electrophoresis.
3. Purify the PCR products using a gel purification kit.
4. Quantify the purified PCR products to use ligation reaction.</td></tr>
<tr><td colspan="3">TA Cloning</td></tr>
<tr><td>2</td><td> 
Thawing materials</td><td>5. Take out the pBHA-T vector and AccuRapid™ 2X Reaction buffer from the kit and thaw them on ice.</td></tr>
<tr><td>3</td><td> 
Preparation of ligation reaction mixture</td><td>6. Prepare the ligation reaction mixture.
• Preparation of ligation reaction mixture
<table><tr><th>Components</th><th>Positive</th><th>Sample</th></tr><tr><td>pBHA-T vector (25 ng/μl)</td><td>2 μl</td><td>2 μl</td></tr><tr><td>PCR products</td><td>-</td><td>0.5-2 μl*</td></tr><tr><td>Control Insert (70 ng/μl)</td><td>1 μl</td><td>-</td></tr><tr><td>AccuRapid™ 2X Reaction buffer</td><td>5 μl</td><td>5 μl</td></tr><tr><td>T4 DNA Ligase (200 U/μl)</td><td>1 μl</td><td>1 μl</td></tr><tr><td>Nuclease-free water</td><td>1 μl</td><td>Variable</td></tr><tr><td>Total volume</td><td>10 μl</td><td>10 μl</td></tr></table>* Note: In general, 0.5-2 μl of PCR product is used, and it is recommended to add in a molar ratio of 5 : 1 (insert: vector) or higher for a more efficient ligation reaction.
7. Mix the reaction mixture by tapping and briefly spin down.</td></tr>
<tr><td rowspan="2">4</td><td colspan="2">• Perform transformation using heat-shock or electroporation</td></tr>
<tr><td> 
Ligation and transformation</td><td>8. Incubate the ligation reaction mixture for at least 15 min at 25°C. For large amounts of transformants, incubate the reaction mixture for 1 hr.
9. Spread the transformation mixture on the LB agar plates containing ampicillin, X-Gal, and IPTG.</td></tr>
<tr><td>5</td><td> 
Analysis</td><td>10. Pick at least 10 white colonies and perform colony PCR.
11. Analyze the bands using electrophoresis.</td></tr>
</table>

Copyright 2022 BIONEER Corporation. All Rights Reserved.