SuperPhi RCA Kit
With Specific Primers

Cat#: Phi100S or Phi1000S

This kit was developed to amplify specific DNA using customized primers.

FOR RESEARCH USE ONLY

SuperPhi RCA Kit is intended for molecular biology use and *in vitro* use only. This product is not intended for diagnosis, prevention or treatment of a disease in human beings or animals.

Store Kit at <-20°C on Receipt

Introduction

This kit was developed to amplify specific template DNA using customized primers through Rolling Circle Amplification (RCA) technology. The starting circle DNA template concentration should be more than one nano-gram. Using excessive multiple primers complementary to template DNA in RCA reaction will generate multiple amplification origins and significantly enhance DNA amplification efficiency.

Kit Components:

Components	100 Reactions (Cat#: Phi100)	1000 Reaction (Cat#: Phi1000)	Storage
Sample Buffer	800 µl	**10x 800 µl**	**-20°C**
Reaction Buffer C*	300 µl	**10x 300 µl**	**-20°C**
SuperPhi Enzyme	100 µl	**10x 100 µl**	**-80°C**

*: Users need add specific primers (final concentration: 10 µM) to reaction.

Detailed Protocol

1. Preparation of Sample Mix:

Sample Mix could be prepared, depending on material sources, as described below:

1.1. Purified DNA:

Transfer 3 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 1 µl of circular DNA (≥ 1 pg/µl) to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.2. Bacterial colonies:

Transfer 4 µl of Sample Buffer into a 0.2 ml PCR tube.
Pick 1/10 to 1/100 of the colony (approximately 10^2–10^4 cells) and add to the above PCR Tub.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.3. Liquid bacterial culture:

Transfer 3.5~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~0.5 µl of saturated overnight culture to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.4. Glycerol stock:

Transfer 3.5~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~0.5 µl of glycerol stock to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

Note: *Heating at higher temperature or longer time may increase the probability of releasing host genomic DNA into cell lysis to the reaction, where the host genomic DNA will compete with the desired template DNA during amplification.*

2. DNA Amplification:

Mix 3 µl of Reaction Buffer C, 2 µl of Specific primer (50 µM) and 1 µl of SuperPhi Enzyme and add to the above PCR tube contains 4 µl of Sample Mix as showed below:

Component	Volume/Reaction
Sample Mix	4 µl
Reaction Buffer C	3 µl
Specific Primers (50 µM)	2 µl
SuperPhi Enzyme	1 µl

One can prepare the master mix of Reaction Buffer and SuperPhi Enzyme for multiple reactions. The final volume is 10 µl. Mix well and incubate at 30 ˚C for 3~24 hrs.

3. Inactivate SuperPhi: Inactivate the enzyme by incubating at 65 ˚C for 10 min, and then cool to 4 ˚C.

4. Perform Downstream Application:

The sample can be examined by agarose gel or applied for cloning and cycle sequencing. An aliquot of the amplified DNA can be directly added into the cycle sequencing reaction without further purification. Specially, the amplified RCA products as concatemers can be used to transform *Bacillus* directly. Compared to the traditional *Bacillus* transformation methods, the SuperPhi product would give the highest transformation rate, which is of critical importance to *Bacillus* gene cloning, expression and library construction.

5. FAQ and Troubleshooting: Please contact us at info@evomicscience.com.