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SuperPhi RCA Premix with Specific Primers

Cat#: PM100S or PM1000S

FOR RESEARCH USE ONLY

SuperPhi RCA Pre-mix 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

Description

This kit was developed to amplify specific template DNA using customized primers through Rolling Circle Amplification (RCA) technology. This premix is optimized for efficient and reproducible RCA, an equivalent of *SuperPhi* RCA with Specific Primers Kit (Cat#: Phi100S). This premixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for RCA set up. 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#: PM100)	1000 Reaction (Cat#: PM1000)	Storage
Sample Buffer	800 µl	10x 800 µl	-20°C
SuperPhi Premix with Specific Primers (#PM100S)	500 µl	10x 500 µl	<-20°C

Detailed Protocol

1. Preparation of Sample Mix:

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

1.1. Purified DNA or DNA ligation/assembly reactions:

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 3.0~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~1.0 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 3.0~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~1.0 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 nicking target DNA and 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:

Add 5 μl of SuperPhi Premix (#PM100S) and 1 μl of specific primer(s) to the above PCR tube contains 4 μl of Sample Mix as showed below:

Component	**Volume/reaction**
Sample Mix with denature DNA	4 μl
SuperPhi Premix with Specific Primers (#PM100S)	5 μl
Specific Primer (100 μM)	1 μl
Final Volume	**10 μl**

Mix well and incubate at 30 ˚C for 3~24hrs.

3. Inactivate SuperPhi: Incubating at 65 ˚C for 10 min, and then cool to 4 ˚C.

4. Perform Downstream Application:

- The amplified DNA can be directly used for the cycle sequencing reaction without purification;
- The amplified DNA can be directly used for DNA restriction enzyme digestion;
- An aliquot of the amplified DNA can be examined by agarose gel;
- Specially, the amplified RCA products as concatemers, maybe after diluted, can be used to directly transform *Bacillus*. Compared to the traditional *bacillus* transformation methods, the RCA1.0 products 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.**

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