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FemtoPhi™ RCA Premix Kit with Random Primers (2x)

Cat#: FM100 or FM1000

FOR RESEARCH USE ONLY

FemtoPhi RCA Premix 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

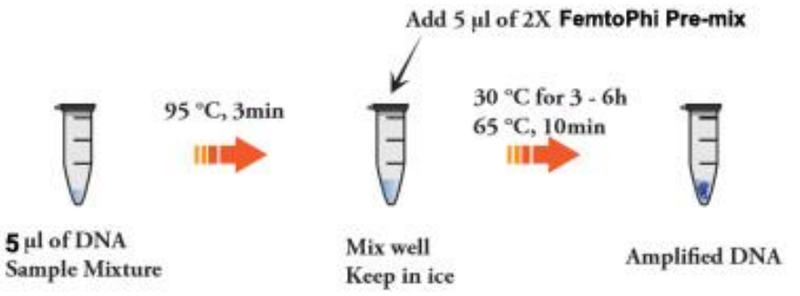
FemtoPhi RCA Premix Kit (Cat#: FM100) is a 2x version premix of FemtoPhi RCA kit, containing all components necessary for a successful and reliable **Rolling Circle Amplification (RCA)**, such as *FemtoPhi* DNA Polymerase, dNTPs, and random primers etc, except circular DNA template. This premixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for RCA set up. The premix is optimized for efficient and reproducible RCA, an equivalent of *FemtoPhi* DNA Amplification (RCA) Kit (Cat#: FP100). *FemtoPhi* RCA Premix Ki has capacity to detect 10 femtogram of plasmid or genomic DNA molecules in 10 microliter of reaction, which is almost 1000 fold sensitive over wild type Phi29 enzyme. Remarkably, the sensitivity of DNA detection using *FemtoPhi* is comparable to PCR or qPCR. Therefore, *FemtoPhi* would be an ideal enzyme to specifically amplify single cell genomic DNA and low concentration DNA from clinical or environmental samples.

Kit Components:

Components	100 Reactions (Cat#: FM100)	1000 Reaction (Cat#: FM1000)	Storage
Sample Buffer	800 µl	10x 800 µl	-20°C
2x FemtoPhi Premix	500 µl	10x 500 µl	<-20°C

Detailed Protocol

FemtoPhi RCA Pre-mix Kit has capacity to detect as low as 10 femtogram of template DNA under optimal condition. 0.2~0.5 µl of saturated overnight culture or 1/10 to 1/100 of the colony (approximately 10^2 ~10^4 cells) would be enough for FemtoPhi RCA reaction. Please keep in mind that excess culture or colony will inhibit FemtoPhi RCA reaction! The protocol described below is a general protocol for amplifying circular DNA from various sources. Yields and kinetics may vary if crude or un-quantified samples are amplified. We recommend to consider a starting point for adapting your specific reaction. Procedures of *FemtoPhi* RCA reaction were outlined below.



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 4 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 5 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 4.5~4.8 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 4.5~4.8 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 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 *FemtoPhi* pre-mix to the above PCR tube contains 5 µl of Sample Mix as showed below:

Component	**Volume/reaction**
Sample Mix with denatured DNA	5 µl
FemtoPhi Premix	5 µl
Final Volume	**10 µl**

Mix well and incubate at 30 ˚C for 3~24 hrs.
Note: Actually, mixing any equal volume of sample mix and FemtoPhi Pre-mix will work fine.

3. Inactivate FemtoPhi: 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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