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FemtoPhi RCA Kit
With Specific Primers

Cat#: FP100S or FP1000S

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

FOR RESEARCH USE ONLY

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

Store Kit at <-20°C on Receipt

This kit was developed to amplify specific DNA using customized primer(s). 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#: FP100S)	1000 Reaction (Cat#: FP1000S)	Storage
Sample Buffer	600 µl	10x 600 µl	-20°C
Reaction Buffer FC*	300 µl	10x 300 µl	-20°C
FemtoPhi Enzyme	200 µl	10x 200 µl	-80°C

***User should add specific primers (final concentration 10 µM) to reaction.**

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 3.5~3.8 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 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 3 µl of Reaction Buffer FC, 1 µl of Specific Primer, and 2 µl of FemtoPhi Enzyme to the above PCR tube contains 4 µl of Sample Mix as showed below:

Component	**Volume/Reaction**
Sample Mix with denatured DNA	4 µl
Reaction Buffer FC	3 µl
Specific Primers (100 µM)	1 µl
FemtoPhi Enzyme	2 µl
Final Volume	10 µl

The final volume is 10 µl. Mix well and incubate at 30 ˚C for 3~24 hrs.
One can prepare the master mix of Reaction Buffer FC, Specific Primers, and FemtoPhi Enzyme if multiple reactions will be run.

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 can be used to transform *Bacillus* after diluted or purified. Compared to the traditional *Bacillus* transformation methods, the FemtoPhi product would give the highest transformation rate, which is of critical importance to *Bacillus* gene cloning, expression and library construction. Please note that, the FemtoPhi product cannot mix with *Bacillus* without further dilution or purification due to its toxicity to the cell. If you do need large amount of RCA product for *Bacillus* transformation, please follow the protocol of "Purification of RCA product" described below. Alternatively, we recommend our PicoPhi DNA Amplification Kit (Cat#: Phi100) which does not contain the toxic components, and the PicoPhi product can be directly transformed to *Bacillus*, without additional handling.

5. Option: Purification of RCA product

1. Please use the following protocol, in case you need further purify RCA product prior to usage.

2. Mix equal volume of FemtoPhi product with PEG-NaCl solution (20% PEG8000 in 2.5M NaCl) in a 1.5ml tube; tapping the tube until the white cloudy precipitate comes out from the solution. Incubation at 37 ˚C for 15min if you didn't see the precipitate.
3. Spin for 1 min to pellet the DNA and remove the supernatant completely but not disturb the pellet; wash the pellet by 500 µl PEG-NaCl once.
4. Dissolve the pellet by at least one volume of TE buffer or water; tap and sit at room temperature or 37 ˚C overnight to dissolve. No pipette, no vertex.

6. FAQ and Troubleshooting: Please contact us at info@genexp-llc.com.

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