
PicoPhi DNA Amplification (RCA) Kit with Random Primers

Cat#: Phi100 or Phi1000



FOR RESEARCH USE ONLY

PicoPhi DNA Amplification 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

Rolling Circle Amplification (RCA) is an isothermal enzymatic process, which amplifies circular or genomic DNA by a Phi29 DNA polymerase-based rolling circle mechanism in the presence of the short DNA or RNA primers, as shown in Fig. 1. Phi29 DNA polymerase is a monomeric protein with two distinct functional domains, C-terminal DNA polymerase domain for DNA synthesis and a spatially separated N-terminal domain with a 3'-5' exonuclease activity for proofreading activity. Co-operation as well as delicate competition between two functional domains, ensure the accurate and efficient DNA synthesis at an optimal rate. Furthermore, this enzyme has capacity to strongly bind to single stranded DNA of double stranded nucleic acid, which makes it favor for multiple displacement amplification (MDA), through debranching double stranded DNA. RCA technology is better than PCR-based methods for DNA amplification because of the high processivity and proofreading activity of Phi29 polymerase. Specially, when genomic DNA is amplified, RCA generates larger fragments (more than 10 kb) with better coverage and less amplification bias, than PCR-based approaches. The RCA technology is so simple, powerful, and versatile that it has been extensively used for DNA cloning, sequencing, SNP and STR genotyping, and genomic DNA amplification, etc. Since the amplified DNA is a concatemer containing tens to thousands of tandem repeats, RCA technology has been employed to generate complex but fine DNA nanostructures, such as DNA origami, nanotubes, and nanoribbons, through manipulating the circular tailor-designed template. These DNA nanostructures have critical potentials in biomedical research, disease diagnostics and therapeutics.

Through optimizing RCA reaction condition and engineering Phi29 enzyme, two classes of RCA DNA amplification technologies, RCA 1.0 and RCA 2.0 as described below, were developed for the various applications. PicoPhi DNA Amplification Kit, a type of RCA 1.0 technology, was suitable for DNA cloning, direct DNA sequencing, and regular DNA amplification; while RCA 2.0 technology would be specifically desired for circle DNA/RNA detection, single cell genomic amplification, etc.

PicoPhi DNA Amplification Kit, based on RCA 1.0 Technology:

Our PicoPhi DNA Amplification Kit (RCA 1.0) was developed to rapidly amplify circular template DNA. This amplification produces microgram quantities of DNA from less than picograms of starting material within 4 hrs at 30°C. Template DNAs can be low or high copy plasmid from bacterial colony, liquid culture, glycerol stock, DNA from BAC culture, M13 plaque, M13 Phage culture supernatant, fosmids or any lambda vectors, DNA ligation/assembly reactions etc. This amplification technology also eliminates the need for overnight cell culture and conventional plasmid or M13 preparations. The amplified



DNA, as high molecular weight and double-stranded concatemers of the circular template, can be used for cloning and direct DNA sequencing without further purification. Specially, the amplified RCA products as concatemers, after diluted, can be used to directly transform *Bacillus*. Compared to the traditional *bacillus* transformation methods, PicoPhi RCA products would give the highest transformation rate, which is of critical importance to *bacillus* gene cloning, expression and library construction. Our highly pure PicoPhi enzyme in PicoPhi DNA Amplification Kit ensures that the amplified DNAs are from your template DNA, but not from the host or environmental DNAs, which are usually found in other Phi29-based DNA amplification kits (Fig.1).

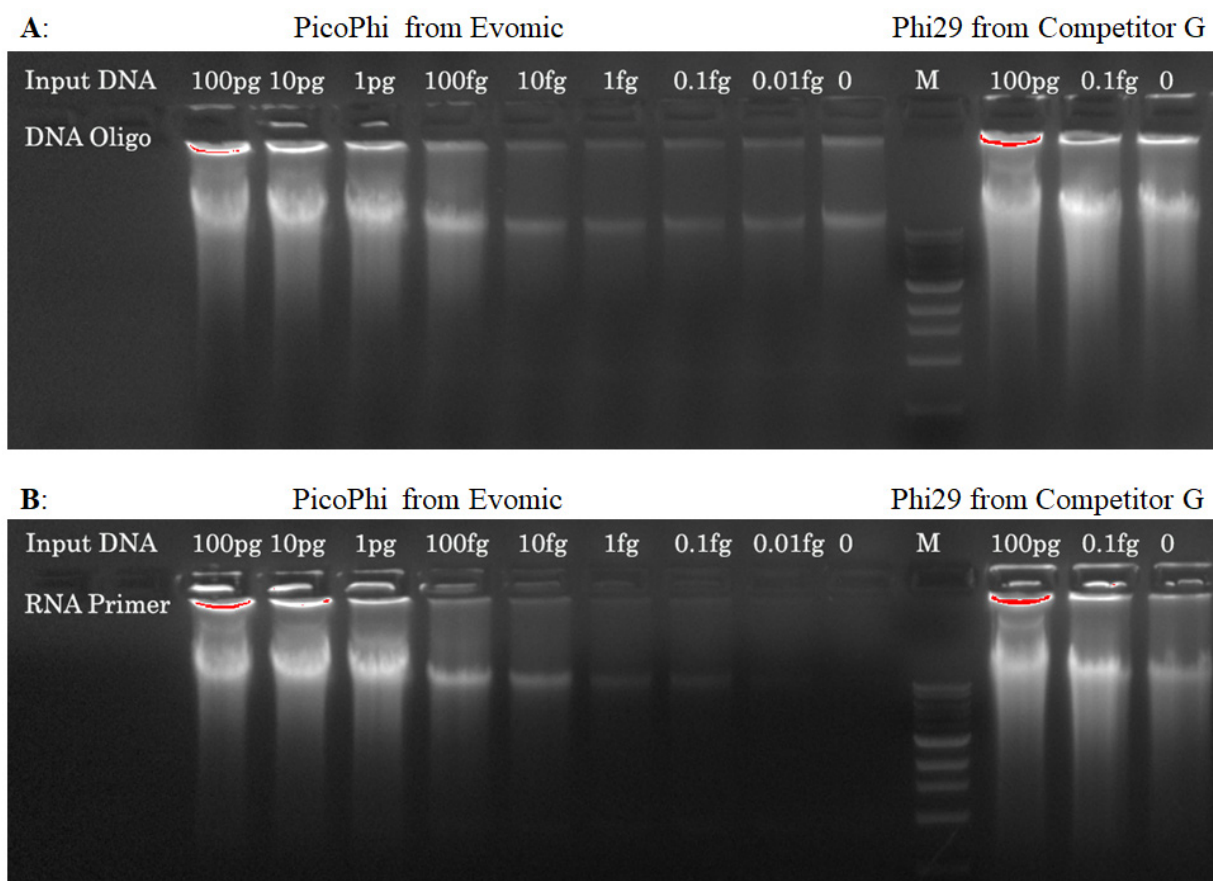


Fig.1. Comparison of purity and activity of our PicoPhi enzyme with Phi29 from competitor G. The indicated amount of denatured pUC19 were mixed with random DNA primer (A) and random RNA primer (B) in the presence of PicoPhi enzyme and Phi29 enzyme from competitor G. After 4 hrs at 30°C, the reaction was stopped and loaded on agarose gel. The intensity of 15 kb amplified product using PicoPhi did response well to the input dosage of pUC19, while that in competitor G did not (in A and B). Even on condition of no input pUC19, the reaction containing Phi29 from competitor G still produced amplified DNA when using RNA primer (in B). That indicated that competitor Phi29 enzyme was contaminated with foreign DNA.



Features of PicoPhi DNA Amplification Kits

- PicoPhi29 DNA polymerase for isothermal multiple displacement amplification (MDA);
- Clean manufacturing process ensures components are free of detectable DNA contamination;
- Greater accuracy than existing other PCR based MDA methods;
- Ease with minimal handling time: 15~20 min for up to 96 samples;
- Easily automated;
- Yield up to 2 µg of highly pure DNA.
- Able to detect down to 1 picogram of circular DNA or genomic DNA.
- Any circle DNA:
 - low or high copy plasmid from bacterial colony,
 - liquid culture and glycerol stock,
 - DNA from BAC culture, M13 plaque, M13 Phage culture supernatant,
 - Fosmids or any lambda vectors,
 - DNA ligation/assembly reactions.
- No further purification of amplified DNA: Direct sequencing and cloning reaction.
- Better results for sequencing than traditional sequencing.
- Compatible with other sequencing kits.
- No need for growing liquid bacterial cultures.

Handling

This kit is used to amplify picogram of DNA. Any contaminated DNA may deteriorate your result. Always wear gloves, and prepare the reaction in a laminar flow hood or similar device to avoid contaminations. Use molecular biology grade clean reagents, sterile reaction tubes and DNA-free pipette tips. Thaw Sample Buffer, Reaction Buffer and PicoPhi Enzyme on ice and keep them in ice all the time.

Quality Control

Each batch of PicoPhi DNA Amplification Kit is tested in-house to ensure consistent product quality. Enzymes used in the kit have been tested separately to ensure the highest purity with no DNA contamination.

The amplified DNA by RCA1.0 technology is suitable for:

- Direct DNA restriction enzyme digestion without further purification;
- Direct DNA sequencing (Sanger's method) without further purification;
- Essential for *Bacillus* plasmid transformation.



PicoPhi DNA Amplification Kits are available for both 100 and 1000 reactions.

Reagent and Equipment to be Supplied by the Users

- Sterile vials, pipettes and pipette tips
- Microcentrifuge
- Cold block
- Sterile, ideally DNA-free certified 0.2 ml PCR tubes
- Thermocycler
- Vortexer

Kit Components:

Components	100 Reactions (Cat#: Phi100)	1000 Reaction (Cat#: Phi1000)	Storage
Sample Buffer	800 µl	10x 800 µl	-20°C
Reaction Buffer	500 µl	10x 500 µl	-20°C
PicoPhi Enzyme	100 µl	10x 100 µl	-80°C

Detailed Protocol

PicoPhi DNA Amplification Kit has capacity to detect as low as 1 pictogram 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 PicoPhi DNA Amplification reaction. Please keep in mind that excess culture or colony will inhibit PicoPhi DNA Amplification reaction! The protocol described below is a general protocol for amplifying circular DNA from various sources. Yields and kinetics may vary if crude or unquantified samples are amplified. We recommend to consider a starting point for adapting your specific 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 5 µl of Reaction Buffer (**Vortex before use**) and 1 µl of PicoPhi Enzyme to the above PCR tube contains 4 µl of Sample Mix as showed below:

Component	Volume/reaction
Sample Mix	4 µl
Reaction Buffer	5 µl
PicoPhi Enzyme	1 µl
Final Volume	10 µl

One can prepare the master mix of Reaction Buffer and PicoPhi Enzyme for multiple reactions. The final volume is 10 µl. Mix well and incubate at 30 °C for 3~124 hrs.

3. **Inactivate PicoPhi:** 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@genexp-llc.com.

Limited Use License and Warranty

Use of this Product is subject to the following terms and conditions. Genxp LLC ("We") warrant that the Product meets the specifications described in this manual. If the terms and conditions are not acceptable, please return all components of Products with original receipt by the original buyer to Genxp LLC within 10 business days. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. No right or license to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Genxp LLC disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein. If you should have any questions or concerns about any of our products, please contact us at info@genexp-llc.com.