PHI29 ROLLING CIRCLE AMPLIFICATION KIT

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-SGR-012
Product Name	Phi29 Rolling Circle Amplification Kit
Category	Template Amplification
Pack Size	50 rxn, 200 rxn
Regulatory Status	For Research Use Only (RUO)
OEM Reference	Contact techsupport@enzovera.com
Version	1.0
Issue Date	2026-05-07

2. INTENDED USE

The Enzovera Phi29 Rolling Circle Amplification Kit is designed for isothermal amplification of circular DNA templates including plasmids, bacterial artificial chromosomes (BACs), fosmids, and circular PCR products. This kit enables direct amplification from picogram quantities of purified circular DNA to microgram quantities in 4–6 hours at 30°C, eliminating the need for bacterial transformation and miniprep prior to Sanger sequencing or other downstream applications. The highly processive Phi29 DNA polymerase provides strand displacement activity and 3' to 5' exonuclease proofreading for accurate amplification of templates up to 100 kb. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Phi29 DNA Polymerase (10 U/µL)	50 µL (50 rxn) / 200 µL (200 rxn)	-20°C
10× Phi29 Reaction Buffer	1 mL (50 rxn) / 4 mL (200 rxn)	-20°C
dNTP Mix (10 mM each)	200 µL (50 rxn) / 800 µL (200 rxn)	-20°C
Random Hexamer Primers (50 µM)	100 µL (50 rxn) / 400 µL (200 rxn)	-20°C
Exonuclease I (20 U/µL)	25 µL (50 rxn) / 100 µL (200 rxn)	-20°C
Exonuclease III (100 U/µL)	25 µL (50 rxn) / 100 µL (200 rxn)	-20°C
Nuclease-Free Water	2 mL (50 rxn) / 8 mL (200 rxn)	Room temperature (15-25°C)

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Thermal cycler or heat block capable of 30°C and 65°C
- Nuclease-free water for dilution of reagents and samples
- Microcentrifuge tubes (0.2 mL, 0.5 mL, or 1.5 mL)
- Pipettes and sterile aerosol-resistant pipette tips
- Ice bucket with ice for reagent preparation
- Vortex mixer for thorough mixing of components

- Microcentrifuge for brief spin-down of samples
- Template DNA (circular plasmid, BAC, or ssDNA as substrate)

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	Kit: enzyme + buffer + dNTPs
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

PROTOCOL: PHI29 ROLLING CIRCLE AMPLIFICATION KIT

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures. This protocol describes the isothermal amplification of circular DNA templates using Phi29 DNA polymerase for rolling circle amplification (RCA). The amplified product is suitable for downstream Sanger sequencing applications.

PRINCIPLE

Phi29 DNA polymerase, a bacteriophage-derived enzyme with high processivity and strand displacement activity, initiates DNA synthesis from random hexamer primers annealed to circular DNA templates. The enzyme continuously synthesizes DNA in a rolling circle mechanism at 30°C, generating multiple concatenated copies of the circular template. This isothermal amplification bypasses the need for bacterial transformation and plasmid miniprep procedures.

MATERIALS PROVIDED

Phi29 DNA Polymerase (10 U/µL)

10X Phi29 Reaction Buffer

Random Hexamer Primers (50 µM)

dNTP Mix (10 mM each dNTP)

Control Circular DNA Template (10 ng/µL)

Nuclease-Free Water

MATERIALS REQUIRED BUT NOT PROVIDED

Circular DNA template (plasmid, BAC, or circularized PCR product)

Thermal incubator or water bath capable of maintaining 30°C

Thermal cycler or heating block capable of 65°C

Microcentrifuge tubes (0.2 mL or 0.5 mL)

Microcentrifuge

Pipettes and nuclease-free pipette tips

Ice bucket

Vortex mixer

SAMPLE REQUIREMENTS

Input: 1 pg to 100 ng of circular DNA template per reaction

Template must be supercoiled, relaxed circular, or nicked circular DNA

Linear DNA will not amplify efficiently

Template should be free of RNA contamination (RNase A treatment recommended if necessary)

Template should be free of proteins and salts that may inhibit polymerase activity

PROTOCOL

PREPARATION

1. Thaw all kit components on ice except Phi29 DNA Polymerase, which should remain at -20°C until immediately before use.

2. Vortex all components except the enzyme for 5 seconds and centrifuge briefly to collect contents at the bottom of the tubes.

3. Prepare the thermal incubator or water bath and set to 30°C. Verify temperature stability before beginning the reaction.

4. Prepare a heating block or thermal cycler and set to 65°C for the enzyme inactivation step.

REACTION SETUP

5. For each reaction, add the following components to a nuclease-free 0.2 mL or 0.5 mL microcentrifuge tube on ice in the order listed:

Nuclease-Free Water: to final volume of 50 µL

10X Phi29 Reaction Buffer: 5 µL

dNTP Mix (10 mM each): 5 µL (final concentration 1 mM each dNTP)

Random Hexamer Primers (50 µM): 2 µL (final concentration 2 µM)

Circular DNA Template: 1 pg to 100 ng (typical input 10-100 ng for plasmids, 1-10 ng for BACs)

6. Mix gently by pipetting up and down 5 times or by gentle vortexing for 2 seconds. Avoid vigorous mixing.

7. Centrifuge briefly (3-5 seconds) to collect the reaction mixture at the bottom of the tube.

PRIMER ANNEALING

8. Incubate the reaction mixture at 30°C for 5 minutes to allow random hexamer primers to anneal to the circular template.

9. During this incubation, remove the Phi29 DNA Polymerase from -20°C storage and place on ice.

ENZYME ADDITION AND AMPLIFICATION

10. After the 5-minute primer annealing step, place the reaction tubes on ice.

11. Add 2 µL of Phi29 DNA Polymerase (10 U/µL) to each reaction for a final concentration of 0.4 U/µL (20 U total per 50 µL reaction).

12. Mix immediately but gently by pipetting up and down 5 times. Do not vortex after enzyme addition.

13. Centrifuge briefly (3-5 seconds) to collect the reaction mixture.

14. Transfer the reaction tubes to the 30°C incubator or water bath.

15. Incubate at 30°C for 4 to 6 hours without agitation. For optimal yield from low-input templates (less than 10 ng), extend incubation to 6 hours. For higher input amounts (50-100 ng), 4 hours is typically sufficient.

ENZYME INACTIVATION

16. After the amplification incubation, transfer the reaction tubes to a heating block or thermal cycler preheated to 65°C.

17. Incubate at 65°C for 10 minutes to inactivate the Phi29 DNA Polymerase.

18. Cool the

8. EXPECTED RESULTS

Amplification of 1–10 ng circular template DNA typically yields 5–20 µg product after 4–6 hours incubation at 30°C, representing 1000–10,000-fold amplification. Rolling circle products appear as high molecular weight concatemers (>10 kb) on agarose gel electrophoresis and yield high-quality Sanger sequencing reads (>800 bp, QV20) when used directly as template with appropriate sequencing primers. Amplification efficiency is template-dependent, with supercoiled plasmids (2–10 kb) and circularized PCR products yielding optimal results compared to relaxed or nicked circular DNA.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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