phi29 DNA Polymerase

Catalog Numbers EP0091, EP0092, EP0094

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Thermo Scientific™ phi29 DNA Polymerase is a highly processive polymerase featuring strong strand displacement activity, which allows for efficient isothermal DNA amplification.

Produced from *E. coli* cells with a cloned gene 2 of *Bacillus subtilis* phage phi29, phi29 DNA Polymerase possesses 3'–5' proofreading exonuclease activity that acts preferentially on single-stranded DNA or RNA.

Applications for the phi29 DNA Polymerase include:

- Rolling circle amplification (RCA)
- Whole genome amplification (WGA) from:
  - DNA from single cells, uncultured microbial cells and viral particles
  - Metagenomes
  - Genomic DNA for SNP and STR detection
- RNA-primed DNA amplification

Contents and storage

Component	EP0091	EP0092	EP0094	Storage
phi29 DNA Polymerase (10 U/µL)	250 U	1000 U	5000 U	–25°C to –15°C
10X Reaction Buffer for phi29 DNA Polymerase	0.25 mL	1.0 mL	5 × 1.0 mL	

Required materials

- Template DNA
- Nuclease-free water
- Exo-Resistant Random Primer (500 µM) (Cat. No. SO181) or target-specific primers
- dNTP Mix (10 mM each) (Cat. No. R0191)
- 0.2 mL PCR tubes or PCR reaction plates
- PCR thermal cycler or heat block
- DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890)

Optional materials

- Pyrophosphatase, inorganic (0.1 U/µL) (Cat. No. EF0221)
- DTT (Cat. No. R0861)
- Quant-iT™ PicoGreen™ dsDNA Assay Kit (Cat. No. P7589) or Qubit™ dsDNA Quantification Assay Kit (Cat. No. Q32850)
- FastDigest™ restriction enzymes (see **thermofisher.com/fastdigest**)
- E-Gel™ Precast Gel Electrophoresis System (see **thermofisher.com/egel**)
- GeneJET™ Gel Extraction and DNA Cleanup Micro Kit (Cat. No. K0831) or MagMAX™ Pure Bind Beads (Cat. No. A58521)

Important guidelines

- To minimize contamination risk, aliquot kit components into smaller volumes before first use. Using single-use aliquots for experiments is recommended.
- Thaw kit components on ice and keep them on ice during reaction setup.
- Mix all components well and centrifuge the contents down before use to ensure homogeneity.
- Addition of DTT and pyrophosphatase to reaction mixtures containing phi29 DNA Polymerase can enhance DNA amplification and increase product yield.
- DTT is sensitive to changes in temperature so switch to a fresh aliquot if the current aliquot has gone through more than 10 freeze-thaw cycles.

Optimization strategies

Template recommendations

Template	Recommendation
Purified circular DNA	Use 1 fg to 1 ng for a 20 µL reaction
Bacterial samples containing circular DNA (e.g., *E. coli* transformants)	Dilute the liquid bacterial culture or colony sample 10 times in nuclease-free water, then use 1 µL of the diluted sample for the RCA reaction.
Purified linear DNA	Use 1 pg to 1 ng for a 20 µL reaction

Primer recommendations

- phi29 DNA Polymerase possesses strong 3'–5' exonuclease (proofreading) activity. To prevent digestion, primers must be designed with at least two phosphorothioate (PTO) bond modifications on the 3′ end.
- For amplification with random primers, use 50 µM of Exo-Resistant Random Primer (Cat. No. SO181).
- For amplification with target-specific primers, start with a primer concentration of 1.0 µM. To achieve the best results, test different concentrations of primer (0.1–1.0 µM) to determine the optimal concentration for your experiment.

Amplification time

- Optimal amplification time for a DNA amplification reaction is 4 hours.
- For samples with ≥1 pg of DNA input, DNA amplification time can be shortened to 2 hours if maximizing product yield is not a priority.
- For samples with very low DNA input (1 fg of circular DNA), amplification time can be prolonged for up to 16 hours to maximize product yield.

Contamination prevention

Contamination prevention is very important in isothermal amplification. Environment-borne and carry-over contamination can result in non-specific amplification. Observe the following guidelines to minimize the risk of contamination.

- Always follow recommended cleaning instructions prior to and after each experiment (see "Cleaning the workspace" for more details).
- Use aerosol-resistant filtered pipette tips only.
- Change pipette tip after each aspiration/dispensation.
- Change gloves frequently during the experiment.
- Use separate dedicated areas, equipment, and supplies for different stages of the experiment:
  - It is highly recommended that kit components only be handled in a laminar flow hood.
  - Make sure that reaction setup prior to DNA template addition (and NTC samples) is handled in a DNA-free/RNA-free laminar flow hood.
  - Use a different laminar flow hood environment for handling DNA.
  - Use different working areas for reaction setup, the amplification reaction, and subsequent procedures.
- Use a negative control (e.g., no template control) to test for background amplification. Always make sure that tubes or wells with negative controls are sealed well.
- If carry-over contamination is suspected, discard used reagents and replace with new vials.

Note: For more details regarding Good Laboratory Practices (GLP), visit **thermofisher.com**.

Cleaning the workspace

To minimize the risk of environment-borne contamination, clean the laboratory workspace and all equipment thoroughly before and after setting up each experiment. Use the following reagents in the given order:

1. DNA*Zap*™ PCR DNA Degradation Solutions
2. UltraPure™ DNase/RNase-Free Distilled Water
3. 70% ethanol solution

Alternatively, the working area can be cleaned with a 0.5-1% bleach solution.

Perform RCA with phi29 DNA Polymerase

IMPORTANT! To avoid contamination, set up reactions in DNA-free environment. See “Contamination prevention” on page 2 for more information.

1. Prepare reaction mixture on ice by adding the components listed in the table.

   The volumes are provided for a single 20 µL reaction. Multiply the volumes of components common to all reactions by the required number of reactions plus an additional 10% to account for variations in pipetting.

Component	RCA with exo-resistant random primers		RCA with target-specific primers	
	Volume	Final concentration	Volume	Final concentration
10X Reaction Buffer for phi29 DNA Polymerase	2 µL	1X	2 µL	1X
(*Optional*) DTT (0.1 M) [1]	0.6 µL	3 mM	0.6 µL	3 mM
Exo-Resistant Random Primer (500 µM)	2 µL	50 µM	—	—
Exo-resistant target-specific primer (10 µM) [2]	—	—	2 µL	1 µM
dNTP mix (10 mM each)	2 µL	1 mM	2 µL	1 mM
Circular DNA template [3]	*x* µL	1 fg–1 ng	*x* µL	1 fg–1 ng
Nuclease-free water	Fill to 18 µL	—	Fill to 18 µL	—

[1] Addition of DTT to the reaction mixture may increase product yield.
[2] Optimization of primer concentrations might be required to achieve the best performance. See “Optimization strategies” on page 2 for more information.
[3] For RCA directly from bacterial samples, dilute the sample 10 times with nuclease-free water and add 1 µL for a single 20 µL reaction.

2. Gently vortex the mixture, then briefly centrifuge the tube to collect the components from the walls of the tube.

3. Incubate the prepared reaction mix in a thermal cycler at 95°C for 3 minutes, then immediately place the tube back on ice.

4. Add polymerase to the denatured reaction mix.

Reagent	Volume	Final concentration
Denatured reaction mix	18 µL	—
phi29 DNA Polymerase (10 U/µL)	1 µL	10 U/reaction
(*Optional*) Pyrophosphatase, inorganic (0.1 U/µL) [1]	1 µL	0.1 U/reaction

[1] Adding Pyrophosphatase, inorganic (0.1 U/µL) (Cat No: EF0221) to the reaction mixture may enhance DNA amplification and facilitate sample handling. If the enhancement of DNA amplification is not crucial, add 1 µL of nuclease-free water instead of inorganic pyrophosphatase.

5. Gently vortex the mixture, then briefly centrifuge the tube to collect the components from the walls of the tube.

6. Incubate samples in a thermal cycler, set with the following program:

Step	Temperature	Time
Amplification	30°C	4 hours
Inactivation	65°C	10 minutes

7. Store RCA products overnight at 4°C, or at -20°C for long-term storage.

Perform MDA-WGA with phi29 DNA Polymerase

IMPORTANT! To avoid contamination, set up reactions in DNA-free environment. See “Contamination prevention” on page 2 for more information.

1. Prepare reaction mixture on ice by adding the components listed in the table.

   The volumes are provided for a single 20 µL reaction. Multiply the volumes of components common to all reactions by the required number of reactions plus an additional 10% to account for variations in pipetting.

Component	Volume	Final concentration
10X Reaction Buffer for phi29 DNA Polymerase	2 µL	1X
(*Optional*) DTT (0.1 M) [1]	0.6 µL	3 mM
Exo-Resistant Random Primer (500 µM)	2 µL	50 µM
dNTP mix (10 mM each)	2 µL	1 mM
Linear DNA template	*x* µL	1 pg–1 ng
Nuclease-free water	Fill to 18 µL	—

[1] Addition of DTT to the reaction mixture may increase product yield.

2. Gently vortex the mixture, then briefly centrifuge the tube to collect the components from the walls of the tube.
3. Incubate the prepared reaction mix in a thermal cycler at 95°C for 3 minutes, then immediately place the tube back on ice.
4. Add polymerase to the denatured reaction mix.

Reagent	Volume	Final concentration
Denatured reaction mix	18 µL	—
phi29 DNA Polymerase (10 U/µL)	1 µL	10 U/reaction
(*Optional*) Pyrophosphatase, inorganic (0.1 U/µL) [1]	1 µL	0.1 U/reaction

[1] Adding Pyrophosphatase, inorganic (0.1 U/µL) (Cat No: EF0221) to the reaction mixture may enhance DNA amplification and facilitate sample handling. If the enhancement of DNA amplification is not crucial, add 1 µL of nuclease-free water instead of inorganic pyrophosphatase.

5. Gently vortex the mixture, then briefly centrifuge the tube to collect the components from the walls of the tube.
6. Incubate samples in a thermal cycler, set with the following program:

Step	Temperature	Time
Amplification	30°C	4 hours
Inactivation	65°C	10 minutes

7. Store MDA-WGA products overnight at 4°C, or at -20°C for long-term storage.

Postamplification procedures

Guidelines for cleanup and product dilution

- The amplified product may be viscous due to accumulation of high molecular weight DNA. For more accurate quantification and best downstream performance, diluting the product 2–10 times with nuclease-free water prior to analysis or downstream applications is recommended.
- The amplified product can be cleaned up by affinity-based spin column (e.g., GeneJET™ Gel Extraction and DNA Cleanup Micro Kit) or magnetic beads-based purification (e.g., MagMAX™ Pure Bind Beads).

Guidelines for product verification and quantification

- For RCA product, quality can be verified by digestion with restriction enzymes (see “Guidelines for restriction enzyme digestion”), and subsequent analysis by agarose gel electrophoresis. For best results, use the E-Gel™ Precast Gel Electrophoresis System.

- Diluted amplified product can be quantified by using the Quant-iT™ PicoGreen™ dsDNA Assay Kit or Qubit™ dsDNA Quantification Assay Kit in conjunction with the Qubit™ Fluorometer.
- Cleaned amplified product can be quantified using the NanoDrop™ spectrophotometer set at 260 nm of absorbance.

Recommendations for downstream applications

Perform debranching procedure

The following protocol is a general debranching procedure using S1 Nuclease (100 U/µL) (Cat. No. EN0321) performed after DNA amplification.

1. Clean up the amplified product up by affinity-based spin column or magnetic bead-based purification (see "Guidelines for cleanup and product dilution" on page 4).
2. Prepare debranching reaction by adding the components in the following table to a microcentrifuge tube:

Component	Volume	Final Concentration
5X Reaction Buffer for S1 Nuclease	6 µL	1X
S1 Nuclease	10 U	10 U/reaction
Purified amplification product	*x* µL	variable
Nuclease-free water	To 30 µL	—

3. Gently vortex the mixture, then briefly centrifuge the tube to collect the components from the walls of the tube.
4. Incubate the reaction mix at room temperature for 10 minutes.
5. Inactivate the reaction by adding 2 µL of 0.5 M EDTA to the sample and incubating at 70°C for 10 minutes.
6. Clean up the product before downstream use (see "Guidelines for cleanup and product dilution" on page 4).
7. Store samples at -20°C for long-term storage.

Guidelines for restriction enzyme digestion

For best product digestion results, FastDigest™ restriction enzymes are recommended.

- Use RCA product diluted 2–10 times with nuclease-free water as input for digestion following the protocol for the selected restriction enzyme.
- After digestion, analyze the product by agarose gel electrophoresis (see "Guidelines for product verification and quantification")
- If necessary, clean up the digested product before other downstream use (see "Guidelines for cleanup and product dilution" on page 4).

Guidelines for sequencing

- For Sanger sequencing, dilute RCA product 5–20 times with nuclease-free water and prepare the sample according to the respective Sanger sequencing protocol or service provider instructions.
- For next-generation sequencing (NGS), clean up the amplified product prior to the library preparation step and use it according to the respective NGS protocol or service provider instructions (see "Guidelines for cleanup and product dilution" on page 4). The addition of pyrophosphatase does not affect NGS results.

Troubleshooting

Observation	Possible cause	Recommended action
Low product amount or no product	Template DNA quality (e.g., damaged or very low amount of DNA, presence of reaction inhibitors).	Test quality of template DNA before reaction setup.
	Insufficient amplification time.	Increase duration of amplification step (can be increased up to 16 hours).
	DTT degradation.	Replace DTT reagent with a fresh DTT in a new tube.

Observation	Possible cause	Recommended action
Low product amount or no product *(continued)*	Primer design (targetspecific amplification).	Design target-specific primer with at least two phosphorothioate (PTO) bond modifications on the 3´ end.
		Optimize target-specific primer concentration in the range of 0.1–1.0 µM.
False-positive result	Non-specific amplification due to contamination.	Always follow tips from “Important guidelines” on page 2 and “Contamination prevention” on page 2 to minimize contamination risk.
		Perform additional cleaning of working area and equipment if contamination is suspected.
		Replace vials of contaminated reagents with new ones.
Quantification errors	High product yield.	If amplified product remains viscous after initial dilution, increase the dilution of the product and vortex well before using it for quantification measurements.
		Ensure the tested sample is within the detection range of quantification system.

Documentation and support

Customer and technical support

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Revision	Date	Description
B00	16 April 2024	Correction of typo in units.
A.0	8 February 2024	New document for phi29 DNA Polymerase.