

# EquiPhi29™ DNA Amplification Kit

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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](https://www.thermofisher.com/support).

## Product description

The Thermo Scientific™ EquiPhi29™ DNA Amplification Kit is designed for fast and sensitive DNA amplification through rolling circle amplification (RCA) and multiple displacement amplification-whole genome amplification (MDA-WGA) reactions.

Contents of the kit include:

- EquiPhi29™ DNA Polymerase, a proprietary phi29 DNA Polymerase mutant that possesses strong strand displacement activity and ensures high thermostability, reaction speed, product yield, and low amplification bias.
- Optimized reaction buffer, exonuclease-resistant random primers, and dNTPs to provide all the necessary components for DNA amplification.
- Pyrophosphatase to enhance DNA amplification and facilitate sample handling.

Amplified DNA can be used in various downstream applications such as Sanger sequencing, next-generation sequencing, and digestion with restriction enzyme.

## Contents and storage

Component	A65393	A65394	Storage
EquiPhi29™ DNA Polymerase (10 U/μL)	100 μL	5 × 100 μL	-25°C to -15°C
EquiPhi29™ DNA Polymerase Reaction Buffer (10X)	1.0 mL	2 × 1.0 mL	
DTT (0.1M)	250 μL	250 μL	
Exo-Resistant Random Primer (500 μM)	200 μL	2 × 200 μL	
dNTP Mix (10 mM each)	200 μL	1.0 mL	
Pyrophosphatase, inorganic (0.1 U/μL)	100 μL	5 × 100 μL	

## Required materials

- Template DNA (purified DNA or bacterial sample containing DNA of interest)
- Nuclease-free water
- DNAZap™ PCR DNA Degradation Solutions (Cat. No. [AM9890](#))
- PCR thermal cycler or heat block

## Optional materials

- 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](https://www.thermofisher.com/fastdigest))
- E-Gel™ Precast Gel Electrophoresis System (see [thermofisher.com/egel](https://www.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 briefly before use to ensure homogeneity.
- DTT is sensitive to changes in temperature. Switch to a fresh aliquot if the current aliquot has gone through more than 10 freeze-thaw cycles.

## Optimization strategies

### RCA with target-specific primers

- EquiPhi29™ DNA Polymerase possesses strong 3′-5′ exonuclease (proofreading) activity. To prevent primer degradation, primers must be designed with at least two phosphorothioate (PTO) modifications on the 3′ end.
- A starting primer concentration of 1.0 μM is recommended. To achieve the best results, test different concentrations of primer (0.1–1.0 μM) to determine the optimal concentration.
- Use Standalone EquiPhi29™ DNA Polymerase (Cat. No. [A39390](#)) and dNTP Mix (10 mM each) (Cat. No. [R0191](#)) when performing RCA with specific primers.

### Template recommendations

EquiPhi29™ DNA Polymerase amplifies both circular and linear DNA templates, however when performing RCA reactions, the DNA template must be circular.

- Use 1 fg to 1 ng of purified circular DNA as input for a 20 μL reaction.
- If starting from bacterial samples containing plasmid 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.

### Amplification time

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

**IMPORTANT!** To avoid contamination, it is highly recommended to set up reaction mix in a DNA free environment. See “Contamination prevention” on page 2 for more information.

### 1. Add components to the reaction mix on ice.

**Note:** The table provides volumes for a single 20- $\mu$ L reaction. If preparing a master mix, 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.

Reagent	Volume	Final concentration
EquiPhi29™ DNA Polymerase Reaction Buffer (10X)	2 $\mu$ L	1X
DTT (0.1M) <sup>[1]</sup>	0.2 $\mu$ L	1 mM
Exo-Resistant Random Primer (500 $\mu$ M) <sup>[2]</sup>	2 $\mu$ L	50 $\mu$ M
dNTP mix (10 mM each)	2 $\mu$ L	1 mM
Circular DNA template <sup>[3]</sup>	x $\mu$ L	1 fg–1 ng
Nuclease-free water	Fill to 18 $\mu$ L	—

<sup>[1]</sup> For more convenient handling, DTT (0.1 M) can be diluted 10-fold with nuclease-free water prior to reaction setup. If using diluted, use 2  $\mu$ L of the 10 mM DTT for a single 20  $\mu$ L reaction.

<sup>[2]</sup> For RCA with target-specific primer, a primer concentration of 1.0  $\mu$ M is recommended. See “Optimization strategies” on page 2 for more information.

<sup>[3]</sup> For RCA directly from bacterial samples, dilute the sample 10 times with nuclease-free water and add 1  $\mu$ L for a single 20  $\mu$ L 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 and pyrophosphatase to the denatured reaction mix.

Reagent	Volume	Final concentration
Denatured reaction mix	18 $\mu$ L	—
EquiPhi29™ DNA Polymerase (10 U/ $\mu$ L)	1 $\mu$ L	10 U/reaction
Pyrophosphatase, inorganic (0.1 U/ $\mu$ L)	1 $\mu$ L	0.1 U/reaction

### 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	42°C	2 hours
Inactivation	65°C	10 minutes

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

## Postamplification procedures

### Guidelines for cleanup and product dilution

- The RCA 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 RCA 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 RCA 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 RCA product can be quantified using the NanoDrop™ spectrophotometer set at 260 nm of absorbance.

## Recommendations for downstream applications

### Perform debranching procedure

The following protocol describes a debranching reaction using S1 Nuclease (100 U/μL) (Cat. No. [EN0321](#)) that can be performed after an RCA reaction.

1. Clean up the RCA product up by affinity-based spin column or magnetic bead-based purification (see “Guidelines for cleanup and product dilution” on page 3).
2. Prepare debranching reaction by adding the following components 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 RCA product <sup>[1]</sup>	x μL	variable
Nuclease-free water	To 30 μL	—

<sup>[1]</sup> Alternatively, up to 2 μL of untreated RCA product can be used as input.

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 3).
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 3).

### 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 3). 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 4 hours).
	DTT degradation.	Replace DTT reagent with a fresh DTT in a new tube.
	Primer design (target-specific 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 RCA 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

Visit our product page at [thermofisher.com](https://www.thermofisher.com) for additional information and protocols.

For support, visit [thermofisher.com/support](https://www.thermofisher.com/support).

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Revision	Date	Description
A.0	9 February 2024	New document for EquiPhi29 DNA Amplification Kit.

The information in this guide is subject to change without notice.

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