phi29-XT WGA Kit

NEB #E1604S/L

25/100 reactions

Version 1.0_07/24

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Kit Components and Storage Conditions

The kit should be stored at -20°C upon receipt and has a shelf life of 24 months under these conditions. The Neutralization Buffer, phi29-XT Reaction Buffer, Exonuclease-Resistant Random Primers and Deoxynucleotide (dNTP) Solution Mix are stable for at least 30 freeze/thaw cycles. Thaw frozen components at room temperature, and then place all components on ice or at 4°C during use. Store materials at -20°C after use. Each package contains:

Materials for 25 reactions (NEB #E1604S) or 100 reactions (NEB #E1604L)

- Neutralization Buffer
- phi29-XT Reaction Buffer for WGA, 5X
- Exonuclease-Resistant Random Primers, 500 µM
- Deoxynucleotide (dNTP) Solution Mix, 10 mM
- phi29-XT DNA Polymerase for WGA, 10X

Required Materials Not Included:

- DNA template (purified genomic DNA, diluted cells, or sorted cells)
- Denaturation Solution
- Nuclease-free water
- Thin-walled, nuclease-free PCR tubes/plates or microcentrifuge tubes
- Thermocycler

Introduction

Whole Genome Amplification (WGA) with random primers is a method for robust, non-selective amplification of a whole genome sequence. WGA is capable of synthesizing a large amount of genetic information from trace amounts of DNA (microgram quantities of amplified products from femto- or picogram input material), and as such it provides a valuable tool for preserving limited samples in molecular biology. This kit features an engineered phi29 DNA polymerase, phi29-XT. phi29-XT DNA Polymerase generates more DNA product in a shorter amount of time than wild-type phi29 DNA polymerase, while sharing key qualities that are ideal for WGA applications including high processivity, strong strand-displacement activity, and high-fidelity. phi29-XT is also more thermostable than wild-type phi29, with an optimal reaction temperature of 42°C, and has improved sensitivity over wild-type, supporting amplification down to a single cell of DNA input. This kit also includes exonuclease-resistant random primers to universally amplify genomic DNA sequences.

General Tips and Considerations

Input material

- This kit is compatible with extracted genomic DNA or DNA directly from cells.

Primers

- WGA with random primers allows amplification of DNA sequences without needing site-specific primers. WGA performed with random primers will generate branched double-stranded DNA products.
- phi29-XT DNA polymerase, like wild-type phi29 DNA polymerase, has strong 3’ to 5’ exonuclease activity for proofreading. The random primers provided in this kit are exonuclease-resistant by the addition of phosphorothioate bonds to protect them from degradation by phi29-XT. When using target-specific primers rather than the random primers provided in this kit, it is recommended that the primers are protected with a minimum of two phosphorothioate bonds on the 3’ end.

Reaction temperature

- While wild-type phi29 DNA polymerase reactions are typically carried out at 30-37°C, phi29-XT DNA polymerase is more thermostable and works optimally at 42°C. Amplification below 37°C using this kit is not recommended.

Incubation time

- WGA reactions with phi29-XT DNA polymerase should be performed at 42°C for 1.5 hours. For extremely low input amounts (e.g., less than 1 pg genomic DNA), longer incubation times may increase product yield.
- In the absence of template, non-specific DNA products may be produced when WGA reactions are incubated beyond 1.5 hours. However, when a DNA template is present, incubation over 1.5 hours does not typically increase background amplification.

WGA products

- WGA with random primers generates long, branched double-stranded DNA products. These products are suitable for many downstream applications without any further processing steps.
- For applications where the branched nature of the WGA products may be disruptive (e.g., nanopore-based DNA sequencing), debranching using T7 Endonuclease I (NEB #M0302) may be helpful. See the debranching protocol below for details.
- A typical WGA reaction with phi29-XT DNA polymerase will yield >5 µg of DNA from 10 pg of human genomic DNA input in 1.5 hours. Increasing the reaction volume, initial input DNA concentration, or incubation time may increase the product yield.

WGA product quantitation

- WGA products may be directly quantified after dilution by Quant-iT® PicoGreen® dsDNA Assay Kit or Qubit® Fluorometer.
- Purified WGA products can be quantitated by measuring the absorbance at 260 nm or by NanoDrop®.

Protocol for amplification of DNA using the phi29-XT WGA Kit

1. Prepare the Denaturation Solution as indicated below:

COMPONENTS	VOLUME
KOH (1000 mM)	100 µl
EDTA (500 mM)	5 µl
Nuclease free water	895 µl

2. Denature the DNA by mixing 1-4 µl of DNA (<20 ng) or cells (≥1 cell) with 3 µl Denaturation Solution. Incubate at room temperature for 5 minutes.
3. Neutralize the denatured DNA solution by adding 3 µl of Neutralization Buffer, mix well, and place samples on ice.
4. If denatured/neutralized DNA volume is less than 10 µl, bring volume to 10 µl with nuclease-free water.
5. Prepare phi29-XT reaction master mix as described in the table below:

COMPONENTS	SINGLE REACTION	FINAL CONCENTRATION
phi29-XT Reaction Buffer for WGA, 5X	4 µl	1x
Exonuclease-Resistant Random Primers, 500 µM	2 µl	50 µM
Deoxynucleotide (dNTP) Solution Mix, 10mM	2 µl	1 mM
phi29-XT DNA Polymerase for WGA, 10X	2 µl	1x

6. Add 10 µl of phi29-XT reaction master mix to 10 µl of denatured/neutralized DNA. Mix well and centrifuge briefly to collect solutions to bottom of tubes.
7. Incubate in a thermocycler with the lid set at ≥ 75°C, for 1.5 hours at 42°C, followed by 65°C for 10 minutes to inactivate the enzyme.
8. The WGA products can be kept at 4°C overnight or at -20°C for long term storage.

Note: If sample cleanup is necessary, SPRI® beads are recommended, following the manufacturer's protocol. Typically, WGA products can be directly used in downstream applications without cleanup.

General protocols for downstream applications

Debranching protocol

1. Dilute WGA products two-fold with nuclease-free water.
2. Purify WGA products using 0.6X SPRI beads following manufacturer's recommendations.
3. Prepare debranching reactions as described below. Mix well by pipetting, and centrifuge briefly to collect solutions to the bottom of the tube.

COMPONENTS	30 µl REACTION	FINAL CONCENTRATION
Purified WGA products	variable	variable
NEBuffer 2, 10X	3 µl	1x
T7 Endonuclease I	1.5 µl	0.5 units/ µl
Nuclease-free water	to 30 µl	N/A

4. Incubate for 1 hour at 37°C. Debranched product can be further purified by SPRI beads cleanup following manufacturer's protocol.

Next-generation sequencing Library Preparation Protocol

1. Dilute WGA products with nuclease-free water (typically ~50-fold), and measure the concentration of the WGA products by Quant-iT PicoGreen dsDNA Assay Kit or Qubit Fluorometer.
2. Proceed with NGS library prep using the appropriate amount of WGA product. Assemble library according to manufacturer's protocol.

   Note: If proceeding with Illumina sequencing, we recommend using the NEBNext® Ultra™ II FS Library Prep Kit (NEB #E7805).

Troubleshooting guide

Note: For additional assistance please refer to product FAQ's at www.neb.com/E1604.

PROBLEM	POSSIBLE CAUSE(S)	SOLUTIONS
Low product yield	Improper reaction setup	• Perform a positive control reaction using a verified genomic DNA.
	Inhibitor in starting material	• Chemicals in the starting material may inhibit the WGA reactions. Decrease the inhibitor concentration by diluting the starting material in water.
	Low amount of input material	• Increase the amount of input material or the reaction time.
	Contamination present in the reagents or process	• phi29-XT WGA is highly sensitive and efficient. Consequently, be mindful that small amounts of contaminating DNA may also get amplified with your sample. • Follow good laboratory PCR practices. • Replace the contaminated reagent(s).
Nonspecific amplification	Reaction proceeds too long	• If possible, try to limit the reaction to less than 2 h.

Ordering Information

NEB #	PRODUCT	SIZE
E1604S/L	phi29-XT WGA Kit	25/100 reactions

Companion Products

NEB #	PRODUCT	SIZE
N0447	Deoxynucleotide (dNTP) Solution Mix	8/40 µmol
M0302	T7 Endonuclease I	250/1,250 units
M0269	phi29 DNA polymerase	250/1,250 units

Revision History

REVISION #	DESCRIPTION	DATE
1.0	–	07/24