Manual

NxGen phi29 DNA Polymerase

Please read carefully and thoroughly before beginning

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-20 ºC storage required
immediately upon receipt

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Manual

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Contents

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1. Product description

Phi29 DNA Polymerase is normally responsible for the replication of the *Bacillus subtilis* phage phi29[1]. The enzyme is a highly processive DNA polymerase (up to 70,000 base insertions per binding event) with a powerful strand displacement activity[2] and a 3′→ 5′ proofreading exonuclease function[3]. The NxGen phi29 DNA Polymerase is often used for whole genome amplification or wherever a DNA polymerase with strong strand displacement activity is required.

Enzyme source: A recombinant *E. coli* strain carrying the phi29 DNA polymerase gene from bacteriophage phi29.

2. Product specifications

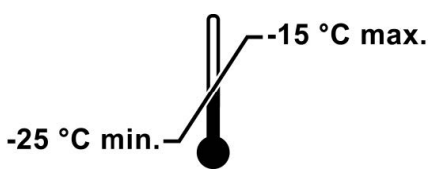
Test	Specification
Unit concentration	10 units/µL
Purity (assessed by SDS-PAGE)	>99%
Single-strand exonuclease activity	Functional/present
Endonuclease activity	<10% converted per 100 units of enzyme
***E. coli* 16S rDNA Contamination**	<10 copies detected per 100 units of enzyme

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
NxGen phi29 DNA Polymerase	2,000 units	30221-1	NxGen phi29 DNA Polymerase	F83900-1	200 µL
			10X phi29 DNA Polymerase Buffer	F88901-1	1.5 mL
	10,000 units	30221-2	NxGen phi29 DNA Polymerase	F83900-1	5 x 200 µL
			10X phi29 DNA Polymerase Buffer	F88901-1	5 x 1.5 mL

4. Storage conditions

Store all kits and components at -20 °C.



5. Materials supplied by the user

- Random hexamer primers (100 µM)
  - Use of random hexamers with 3′ terminal phosphorothioate modifications to prevent exonuclease digestion is recommended (e.g. Cat. no. SO181, Thermo Fisher Scientific)
- Nuclease-free water
- dNTP mix (2.5 mM each dNTP)
- DNA samples
- 0.2 mL thin-walled PCR tubes or 1.5 mL tubes
- Thermal cycler or heat block

6. Before you start

1. Whole genome amplification techniques using phi29 DNA polymerase are sensitive to contaminating DNA. Take extra care when setting up reactions to avoid contaminating reactions with environmental DNA. Work in a covered PCR hood whenever possible.
2. The use of random hexamers with 3′ terminal phosphorothioate modifications is strongly recommended to prevent the degradation of the random hexamers by the 3′ exonuclease activity of phi29 DNA polymerase.
3. Different types of random hexamers are used for whole genome amplification with phi29 DNA polymerase including semi-random or random. In both cases, they should be phosphorothioated at the two bonds on the 3′ end to avoid digestion by phi29 DNA polymerase.
4. LGC, Biosearch Technologies™ has observed better amplification yields when using fully random hexamers that are 3′ phosphorothioated.
5. Biosearch Technologies strongly recommends setting up and running at least one no template control (NTC) to verify that background amplification of contaminating environmental DNA does not occur.
6. The following protocols include a template denaturation step, which has been shown to increase amplified DNA yield. This approach may be skipped if desired.
7. Protocol 1 outlined below utilises a pre-incubation step that exploits the 3′ exonuclease activity of phi29 DNA polymerase to digest any contaminating linear DNA molecules present in an amplification reaction before the addition of sample. Use Protocol 1 when background amplification is observed in your NTC reactions or when performing long reaction incubation times (>4 hours) to amplify samples with very low input amounts.
8. Protocol 2 outlined below is used when background amplification is not an issue or when amplifying more abundant targets and using shorter incubation times (≤4 hours).

7. Protocols

Protocol 1: whole genome amplification using preincubation for background cleanup

1. Denature each template DNA sample by incubating an aliquot of each in a thermal cycler or heat block at 95 °C for 5 minutes.

2. Cool the denatured DNA samples on ice or in the thermal cycler to 4 °C. Centrifuge each tube briefly to collect contents in the bottom of each tube.
3. While the denatured DNA samples are cooling, set up the following cleanup reactions in 0.2 mL PCR tubes for each sample or NTC to be amplified.
   NOTE: *The following reaction formulations are designed for 1 µL of template DNA input. If using a larger volume of input DNA, adjust the volume of nuclease-free water per reaction accordingly.*

	Sample tube	No template control (NTC)
Nuclease-free water	14 µL	14 µL
10X phi29 DNA Polymerase Buffer	2 µL	2 µL
NxGen phi29 DNA Polymerase	1 µL	1 µL
Total volume	17 µL	17 µL

4. Pre-incubate each reaction tube at 30 °C for 30 minutes to allow the NxGen phi29 DNA polymerase enzyme's 3′ exonuclease activity to digest any linear contaminating environmental DNA.
5. Cool each reaction tube on ice and then add the following:

	Sample tube	No template control (NTC)
Random hexamer primers (100 µM)	1 µL	1 µL
dNTP mix (2.5 mM each)	1 µL	1 µL
Denatured template DNA	1 µL	
Nuclease-free water		1 µL
Final reaction volume	20 µL	20 µL

6. Mix each reaction thoroughly by pipetting and then centrifuge briefly to collect contents in the bottom of each tube.
7. Place tubes in thermal cycler or heat block and incubate at 30 °C for 4 to 16 hours.
8. Heat-kill each reaction by incubating at 65 °C for 10 minutes.
9. Visualise amplified products from each reaction by running on a 0.7% agarose gel (100 V, 40 minutes) or equivalent followed by staining with ethidium bromide or similar DNA dye.

Protocol 2: whole genome amplification with no preincubation cleanup

1. Set up each amplification reaction a 0.2 mL PCR tube as follows.
   NOTE: *The following reaction formulations use of 1 µL of DNA template input. If using a larger volume of input template DNA, adjust the volume of nuclease-free water per reaction accordingly.*

	Sample tube	No template control (NTC)
Nuclease-free water	14 µL	14 µL
10X phi29 DNA Polymerase Buffer	2 µL	2 µL
Random hexamer primers (100 µM)	1 µL	1 µL
dNTP mix (2.5 mM each)	1 µL	1 µL
Target DNA (or water for NTC)	1 µL	1 µL
Total volume	19 µL	19 µL

2. Mix each reaction thoroughly by pipetting and then centrifuge briefly to collect contents in the bottom of each tube.
3. To pre-anneal template primer, incubate each reaction in thermal cycler or heat block at 95 °C for 5 minutes, followed by cooling on ice or in the thermal cycler to 4 °C.
4. Mix each reaction thoroughly by pipetting and then centrifuge briefly to collect contents in the bottom of each tube.
5. To each tube, add 1 µL of NxGen phi29 DNA Polymerase and mix by pipetting.
6. Place tubes in thermal cycler or heat block and incubate at 30 °C for 2-4 hours.
7. Heat-kill each reaction by incubating at 65 °C for 10 minutes.
8. Visualise amplified products from each reaction by running on a 0.7% agarose gel (100 V, 40 minutes) or equivalent followed by staining with ethidium bromide or similar DNA dye.

8. Technical support and guarantee

Biosearch Technologies is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the usersupplied reagents are of high quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Product guarantee: Biosearch Technologies guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from date of receipt.

9. Appendix

A: Typical amplification results with no background amplification

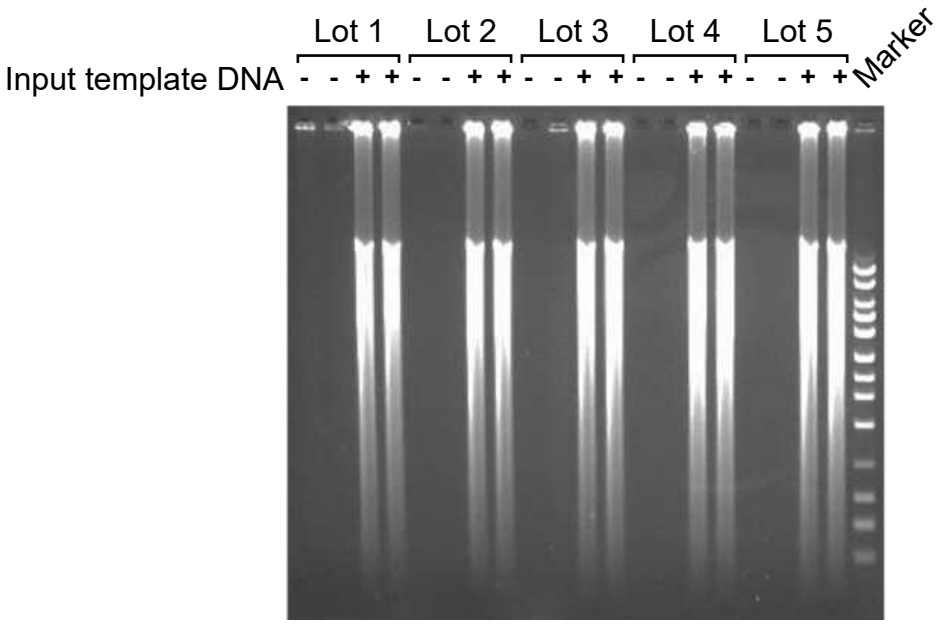


Figure 1. Whole genome amplification with NxGen phi29 DNA Polymerase. 1 ng of human genomic DNA was amplified by whole genome amplification as outlined in Protocol 2 of this manual. Five different lots of NxGen phi29 DNA Polymerase were used and duplicate NTC and (+) human genomic amplification reactions were set up. Amplification reactions were incubated for 4 hours at 30 °C and products were analysed in a 0.7% agarose gel and stained with ethidium bromide to visualise amplified DNA.

B: Demonstration of background amplification with 16 hour incubations

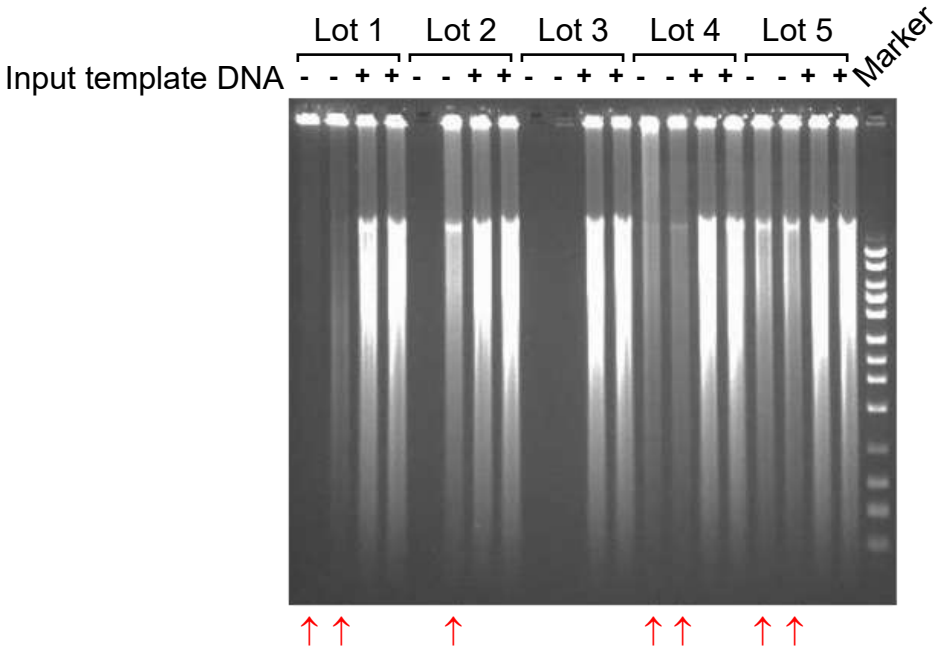


Figure 2. Whole genome amplification with NxGen phi29 DNA Polymerase using 16 hour reaction incubations. 1 ng of human genomic DNA was amplified by whole genome amplification as outlined in Protocol 2 of this manual. Five different lots of NxGen phi29 DNA Polymerase were used and duplicate NTC and (+) human genomic amplification reactions were set up. Amplification reactions were incubated for 16 hours at 30 °C and products were analysed in a 0.7% agarose gel and stained with ethidium bromide to visualise amplified DNA. The red arrows indicate NTC reactions with detectable background (input template independent) amplification.

C: Prevention of background amplification using Protocol 1 with preincubation cleanup

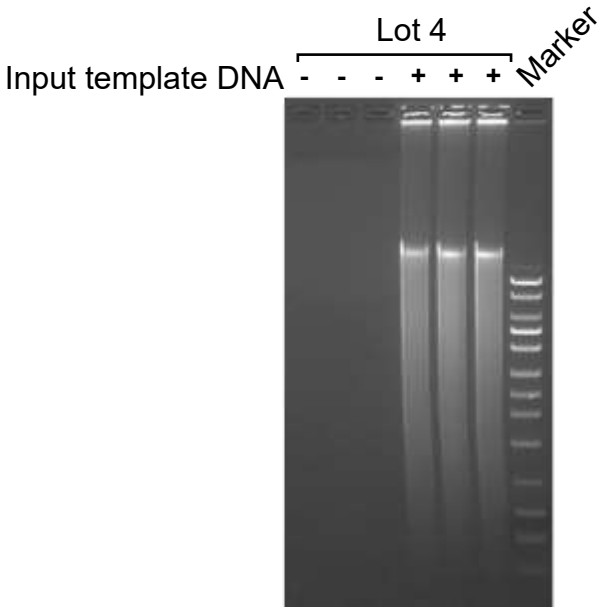


Figure 3 . Whole genome amplification with NxGen phi29 DNA Polymerase using the pre-incubation cleanup step and a 16 hour incubation time. 1 ng of human genomic DNA was amplified by whole genome amplification as outlined in Protocol 1 of this manual. Lot 4 of NxGen phi29 DNA Polymerase was used to set up triplicate NTC and (+) human genomic amplification reactions. Reactions were pre-incubated in the absence of target DNA as outlined in Protocol 1 of this manual followed by incubation for 16 hours at 30 °C. Products were analysed in a 0.7% agarose gel and stained with ethidium bromide to visualise amplified DNA. Note the lack of background amplification in the NTC lanes compared to the background amplification observed for Lot 4 in Figure 2.

D: References and additional reading

1. Blanco L and Salas M 1984 *Proc. Natl. Acad. Sci. USA*, 81, 5325-5329.
2. Blanco L *et al.* 1989 *J. Biol. Chem.,* 264, 8935-8940.
3. Garmendia C *et al.* 1992 *J. Biol. Chem.*, 267, 2594-2599.

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