



NxGen[®] phi29 DNA Polymerase

**Please read carefully and thoroughly
before beginning**

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.



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Technical Support

Lucigen 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 user-supplied 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.

Lucigen Technical Support:

Email: techsupport@lucigen.com

Phone: (888) 575-9695

Product Guarantee: Lucigen 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.

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.

NxGen® phi29 DNA Polymerase

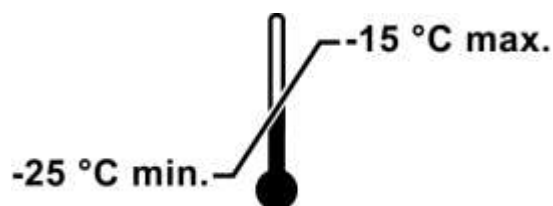
Product Designations and Kit Components

| Product | Size | Catalog Number | Reagent Description | Part Numbers | 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 |

| Product | Size | Catalog Number | Reagent Description | Part Numbers | Volume |
|-----------------------------|--------------|----------------|---------------------------------|--------------|------------|
| NxGen® phi29 DNA Polymerase | 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 |

Components and Storage

Store all kits and components at -20 °C



Product Specifications

| Test | Specification |
|---------------------------------------|---|
| Unit Concentration | 10 U/µL |
| Purity (Assessed by SDS-PAGE) | >99% |
| Single-strand Exonuclease Activity | Functional/Present |
| Endonuclease Activity | <10% converted per 100 units of enzyme |
| <i>E. coli</i> 16S rDNA Contamination | <10 copies detected per 100 units of enzyme |

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 H₂O
- dNTP Mix (2.5 mM each dNTP)
- DNA samples
- 0.2 mL thin-walled PCR tubes or 1.5 mL tubes
- Thermocycler or heat block

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. Lucigen has observed better amplification yields when using fully random hexamers that are 3' phosphorothioated.
5. Lucigen 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 utilizes 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 No Template Control 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).

Protocol 1: Whole Genome Amplification using Pre-incubation for Background Cleanup

1. Denature each template DNA sample by incubating an aliquot of each in a thermocycler or heat block at 95°C for 5 min.
2. Cool the denatured DNA samples on ice or in the thermocycler 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 μ L 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) |
|---------------------------------|-------------------|---------------------------|
| Component | Amount (μ L) | Amount (μ L) |
| Nuclease-free H ₂ O | 14 | 14 |
| 10X phi29 DNA Polymerase Buffer | 2 | 2 |
| NxGen® phi29 DNA Polymerase | 1 | 1 |
| Total Volume | 17 | 17 |

4. Pre-incubate each reaction tube at 30°C for 30 min 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:

NxGen[®] phi29 DNA Polymerase

| | Sample Tube | No Template Control (NTC) |
|---------------------------------|-------------|---------------------------|
| Component | Amount (μL) | Amount (μL) |
| Random hexamer primers (100 μM) | 1 | 1 |
| dNTP Mix (2.5 mM each) | 1 | 1 |
| Denatured template DNA | 1 | |
| Nuclease-free Water | | 1 |
| Final Reaction Volume | 20 | 20 |

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

Protocol 2: Whole Genome Amplification with No Pre-incubation Cleanup

- 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) |
|---------------------------------|-------------|---------------------------|
| Component | Amount (μL) | Amount (μL) |
| Nuclease-free H ₂ O | 14 | 14 |
| 10X phi29 DNA Polymerase Buffer | 2 | 2 |
| Random hexamer primers (100 μM) | 1 | 1 |
| dNTP Mix (2.5 mM each) | 1 | 1 |
| Target DNA (or Water for NTC) | 1 | 1 |
| Total Volume | 19 | 19 |

- Mix each reaction thoroughly by pipetting and then centrifuge briefly to collect contents in the bottom of each tube.
- To pre-anneal template primer, incubate each reaction in thermocycler or heat block at 95°C for 5 min, followed by cooling on ice or in the thermocycler to 4°C
- Mix each reaction thoroughly by pipetting and then centrifuge briefly to collect contents in the bottom of each tube.
- To each tube, add 1 μL of NxGen[®] phi29 DNA Polymerase and mix by pipetting.
- Place tubes in thermocycler or heat block and incubate at 30°C for 2-4 hours.
- Heat-kill each reaction by incubating at 65°C for 10 minutes.

Visualize amplified products from each reaction by running on a 0.7% agarose gel (100V, 40 minutes) or equivalent followed by staining with ethidium bromide or similar DNA dye.

Appendix

A: Typical Amplification Results with No Background Amplification

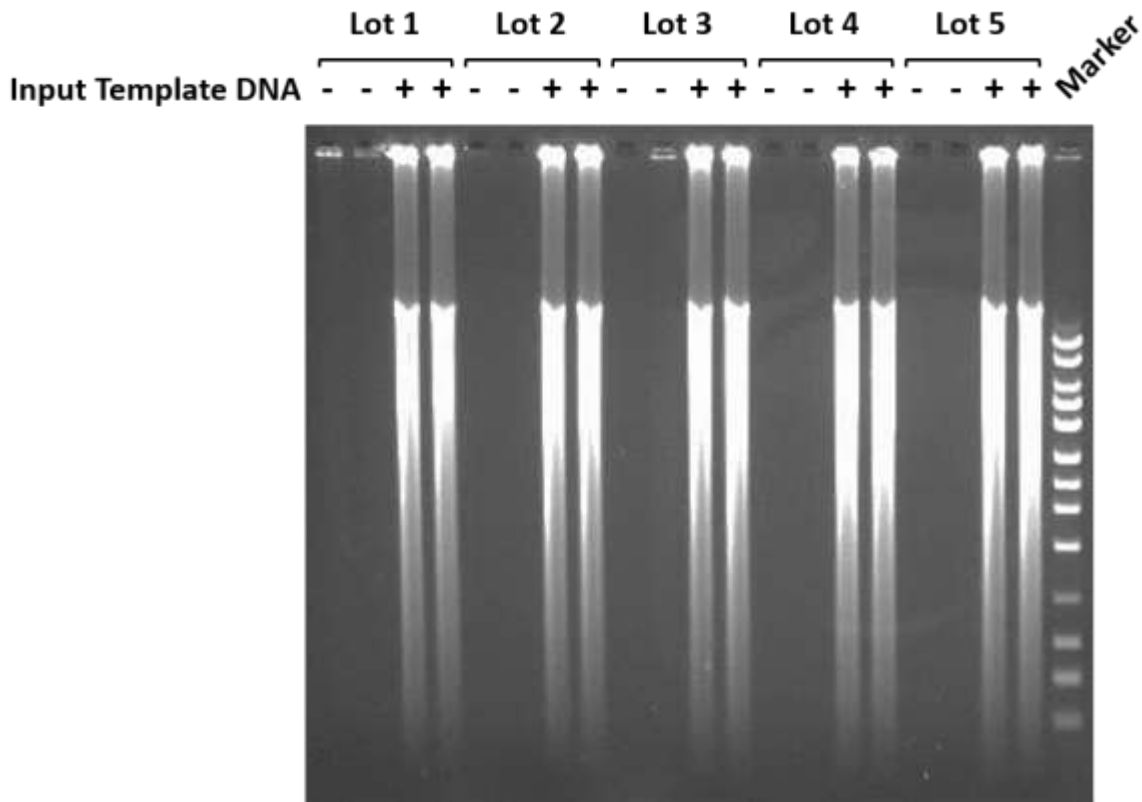


Figure 1. Whole Genome Amplification with NxGen® phi29 DNA Polymerase. 1 ng of human genomic DNA was amplified by whole genome amplification as outlined in Protocol 2 of this manual. Five different lots of NxGen phi29 DNA Polymerase were used and duplicate NTC and (+) human genomic amplification reactions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

B: Demonstration of Background Amplification with 16 Hour Incubations

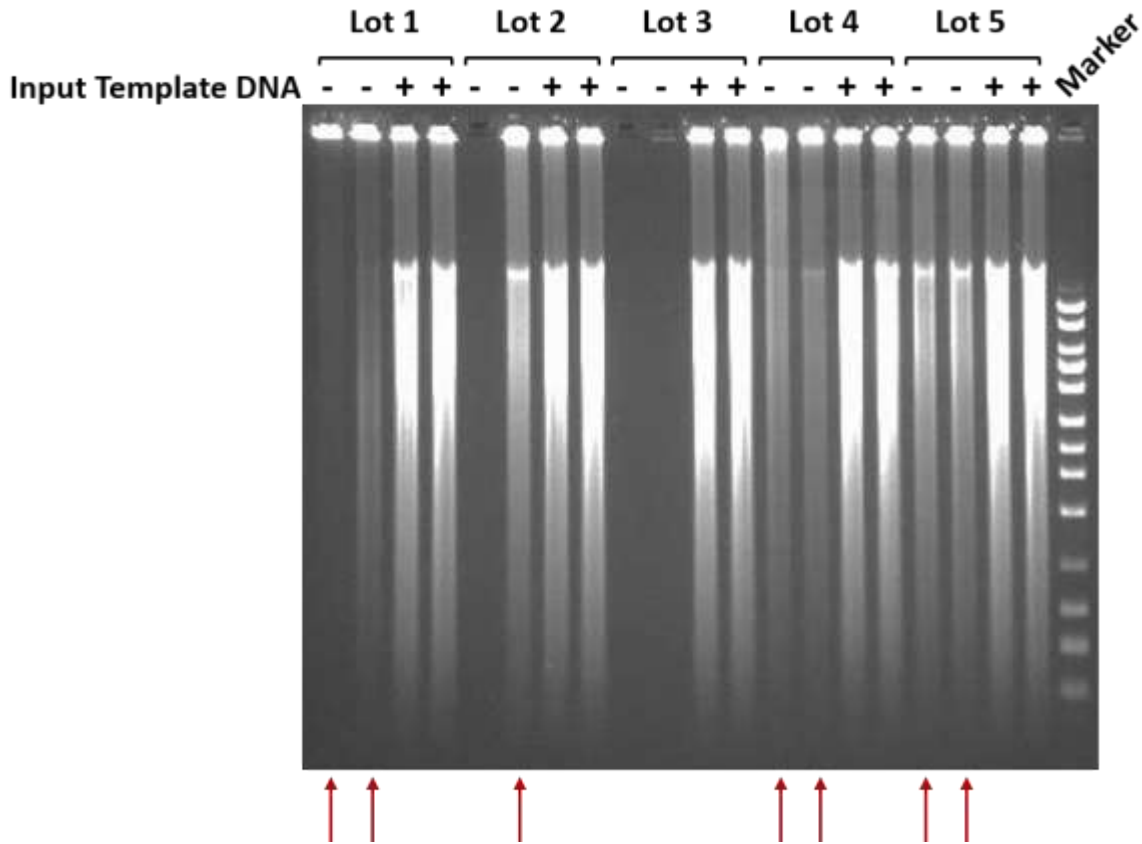


Figure 2. Whole genome amplification with NxGen® phi29 DNA Polymerase using 16 hour reaction incubations. 1 ng of human genomic DNA was amplified by whole genome amplification as outlined in Protocol 2 of this manual. Five different lots of NxGen phi29 DNA Polymerase were used and duplicate NTC and (+)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

C: Prevention of Background Amplification using Protocol 1 with Pre-incubation Cleanup

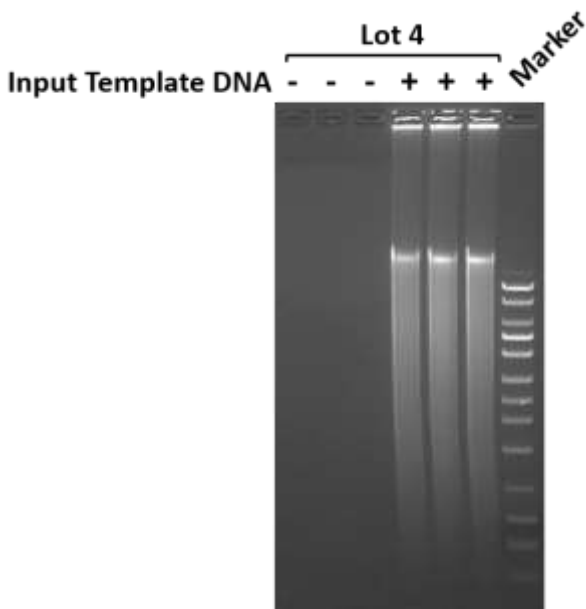


Figure 3. Whole genome amplification with NxGen® phi29 DNA Polymerase using the pre-incubation cleanup step and a 16 hour incubation time. 1 ng of human genomic DNA was amplified by whole genome amplification as outlined in Protocol 1 of this manual. Lot 4 of NxGen phi29 DNA Polymerase was used to set 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 analyzed in a 0.7% agarose gel and stained with ethidium bromide to visualize 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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