# 4BB™ MagniPhi® DNA Polymerase

HANDBOOK

4basebio

# INDEX




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www.4basebio.com

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## INTENDED USE

4BB™ MagniPhi® DNA Polymerase is intended for molecular biology use and *in vitro* use only. This product is not intended for diagnosis, prevention or treatment of a disease in humans or animals.

## ORDERING INFORMATION

| PRODUCT | SIZE | UNITS | CAT. NO. |
|---|---|---|---|
| 4BB™ MagniPhi® DNA Polymerase (10 U/µl) | 25 reactions | 250 | 500025 |
| 4BB™ MagniPhi® DNA Polymerase (10 U/µl) | 100 reactions | 1000 | 500100 |

## PRODUCT DESCRIPTION

4BB™ MagniPhi® DNA Polymerase is the wild type form of Phi29 Polymerase.

Phi29 DNA Polymerase is the monomeric enzyme responsible for the replication of the linear double stranded DNA of bacteriophage phi29 from *Bacillus subtilis* (1). It is an extremely processive polymerase (up to more than 70 kb per binding event) with a strong strand displacement capacity (2). The enzyme displays 3'->5' proofreading exonuclease activity (3), resulting in an extremely high fidelity of synthesis (4). These special features make this enzyme the perfect choice for isothermal DNA amplification or for any application requiring a high fidelity polymerase with strong strand displacement capacity.

## APPLICATIONS

- Highly accurate DNA synthesis (4)
- Rolling Circle Amplification (RCA) (5)
- Whole Genome Amplification (WGA) (6)
- Amplification of DNA from filter paper blood spot samples (7)
- Protein-primed DNA amplification (8)
- RNA-primed DNA amplification (9)
- In situ genotyping with padlock probes (10)
- Recombination based-cloning (11)
- Proximity ligation assay (12).

## REFERENCES

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## KIT CONTENTS

| DESCRIPTION | 25 REACTIONS | 100 REACTIONS |
|---|---|---|
| 4BB™ MagniPhi® DNA Polymerase | 25µl | 100µl |
| 4BB™ MagniPhi® 10x Reaction Buffer | 500µl | 500µl |

## SOURCE

*E.coli* cells harbouring expression construct for 4BB™ MagniPhi® DNA Polymerase.

## ACTIVITY UNIT DEFINITION

One unit of the polymerase catalyzes the incorporation of 1.1 pmol of dAMP onto *Hind*III Φ29 DNA ends in 10 min at 30ºC.

## INACTIVATION

Inactivated by incubation @ 65ºC for 10 min.

## SHIPPING AND STORAGE

4BB™ MagniPhi® DNA Polymerase is shipped on dry ice. On receipt, the kit should be stored immediately at -20ºC in a non frost-free (constant temperature) freezer. When stored correctly, the product can be kept for a minimum of six months after shipping without displaying any reduction in performance. For longer periods, store the kit at -80ºC. Avoid repeated freeze-thaw cycles.

## HANDLING

This kit is sensitive to DNA. Always wear gloves and prepare the reaction in a laminar flow hood or similar device to avoid contaminations. Use molecular biology grade clean reagents, sterile reaction tubes and DNA-free pipette tips. Thaw 4BB™ MagniPhi® DNA Polymerase on ice. Reaction buffer can be thawed at room temperature.

All chemicals should be considered as potentially hazardous. This material may contain substances or activities that are harmful to human health. It should not be ingested, inhaled, or brought into contact with skin, and should be handled with appropriate care in accordance with the principles of Good Laboratory Practice, also mention COSHH. In case of contact with skin wash immediately with water. For more specific information, please consult the Material Safety Data Sheets (MSDS) available on-line at www.4basebio.com.

## QUALITY CONTROL

Absence of endonucleases and exonucleases:

4BB™ MagniPhi® DNA Polymerase has been determined to be free of detectable endonucleases, exonucleases and nicking activity. A fluorogenic substrate designed to react with all these kinds of nucleases has been incubated in the presence of 1µg enzyme for 1 hour at 37°C. No increase in fluorescence compared with the negative control was detected.

Absence of ribonucleases:

4BB™ MagniPhi® DNA Polymerase has been determined to be free of detectable single-strand ribonuclease activity. A fluorogenic substrate designed to react with these kind of nucleases has been incubated in the presence of 1µg enzyme for 30 min at 37°C. No fluorescence increase above the negative control was detected.

Purity:

The purity of the polymerase has been determined to be higher than 95% by SDS-polyacrylamide gel electrophoresis and densitometric measurements.

Functional assay:

4BB™ MagniPhi® DNA Polymerase has been tested for exponential amplification of 10pg and 1ng of high molecular weight human genomic DNA. In the absence of DNA input (non template control, NTC), no background amplification was exhibited under similar conditions.

## REAGENTS AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Sterile vials, pipettes and pipette tips. Use low retention plasticware if possible.
- Sterile, ideally DNA-free certified 0.2ml PCR tubes
- Microcentrifuge
- Cold block
- Thermocycler
- Vortexer
- dNTPs (5mM)
- Tris-HCl pH 7.5 (10mM)
- 3'-protected random hexamer primers (500µM)
- Nuclease-free water.

**Optional:** Quant-iT™ PicoGreen® dsDNA quantification reagent (Invitrogen, P7581), Qubit™ dsDNA HS or BR Assay Kit (ThermoFisher, Q32851 or Q32850) or similar reagents for amplified DNA quantification.

## MULTIPLE DISPLACEMENT AMPLIFICATION PROTOCOL

1. SAMPLE PREPARATION:

   Starting material: High-molecular weight linear DNA or circular DNA. If needed, dilute your DNA sample in Tris-HCl 10 mM (pH 7.5) on ice.

| COMPONENT | VOLUME |
|---|---|
| DNA sample | 2.5µl |
| Random hexamer primers (500µM) | 2.5µl |

   Heat the sample at 95ºC for 3 min. Cool down on ice, centrifuge briefly and store the sample on ice.

2. PREPARATION OF AMPLIFICATION MIX:

   Thaw the reaction components, mix and centrifuge briefly. Keep on ice.

   Prepare the amplification mix by adding the components in the order listed in the table below.

| COMPONENT | VOLUME/REACTION |
|---|---|
| 4BB™ MagniPhi® 10x Reaction Buffer | 2.5µl |
| dNTPs (5 mM) | 2.5µl |
| Nuclease-free water | 14µl |
| MagniPhi® DNA Polymerase | 1µl |

   ***Note: Scale up accordingly (10% excess recommended) when performing several reactions at the same time.***

   Mix by vortexing and store on ice until use.

3. DNA AMPLIFICATION:

Add 20µl of the amplification mix to each sample tube (on ice).

Mix by pipetting and incubate at 30°C for 3 hours. Inactivate the reactions at 65°C for 10 minutes. Cool down to 4°C. Store amplified DNA at 4°C for short term storage or at -20°C for long term storage.

*Note: Incubation time can be increased to 6 hours if higher amplification yields are required.*

4. DNA QUANTIFICATION:

Quantify the samples to determine the amplification yield. Picogreen® or Qubit™ dsDNA HS Assay Kit from ThermoFisher can be used for this purpose. Please follow the manufacturer's recommendations.

PicoGreen® quantification of amplified DNA

This protocol is designed for quantification of double stranded DNA using PicoGreen® reagent.

1. Make a 1:150 dilution of PicoGreen® stock solution in 1x TE (10 mM Tris-HCl pH 8.0; 1 mM EDTA). Each quantification reaction requires 20µl. Example: For 30 measurements add 4µl of PicoGreen® to 596µl 1x TE. Protect the solution from light at all times to avoid photodegradation of the PicoGreen® reagent.
2. Prepare a standard curve using genomic DNA. Prepare a 16µg/ml stock solution of gDNA in 1x TE buffer.
3. Prepare 200µl of 1.6, 0.8, 0.4, 0.2 and 0.1µg/ml of genomic DNA using 1x TE.
4. Transfer 20µl of each DNA standard in duplicate into a 96 well plate labelled A.
5. Dilute each amplified DNA sample 1:100 (2µl sample + 198µl 1x TE) in a 96 well plate labelled B.
6. Place 2µl of the 1:100 DNA sample dilution into the 96 well plate labelled A and add 18µl of 1x TE (dilution 1:1000). Residual 1:100 dilution (plate labelled B) might be stored at -20°C for further analysis.
7. Add 20µl of PicoGreen® dilution to each sample (amplified DNAs and DNA standards) in the 96 well-plate labelled A. Gently shake the plate to mix the samples and reagent.
8. Measure fluorescence in a microplate reader (excitation wavelength ~480nm, emission wavelength ~520nm).
9. Calculate the concentration of the amplification product: Generate a standard curve of fluorescence versus concentration of DNA standards. Determine the concentration of 4BB™ MagniPhi® DNA Polymerase amplified products from the equation of the line derived from the standard curve. Dilution factor during the assay must be taken into consideration when calculating total yields.

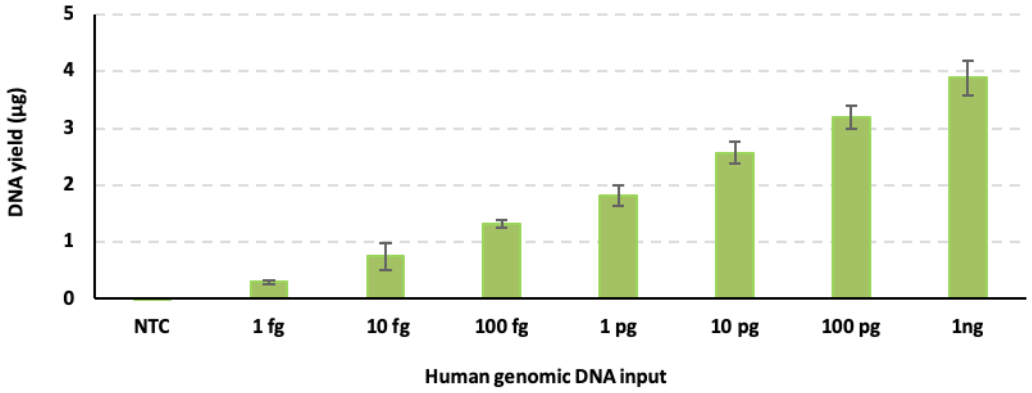
Qubit™ quantification of amplified DNA

We recommend the Qubit™ procedure, as it produces more reliable results in our hands. Please follow the manufacturer's recommendations.

## AMPLIFICATION RESULTS

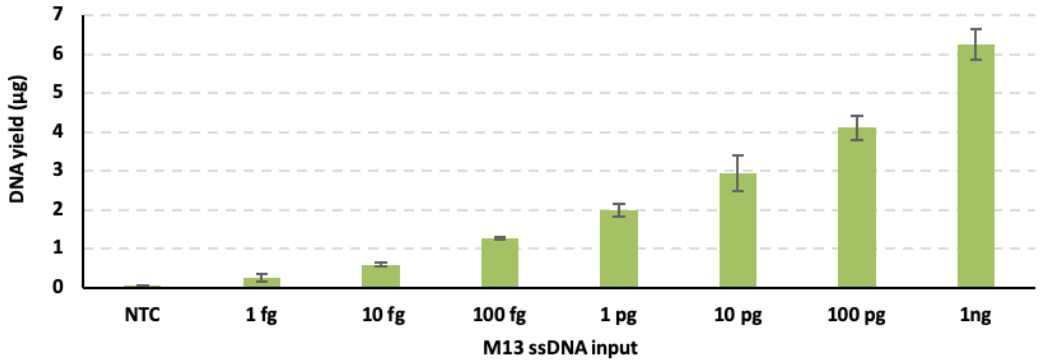
### A. Whole Genome Amplification (WGA) from high molecular weight human genomic DNA

Different DNA doses ranging from 1 fg to 1 ng of human genomic DNA (Promega Ref. G147A) were used as input in the amplification reactions. Input DNA was subjected to the workflow detailed above. Reaction mixtures were incubated for 3 hours at 30ºC and 4BB™ MagniPhi® DNA Polymerase was inactivated for 10 min at 65ºC, to avoid degradation of the amplification products. Amplified DNA was quantified using the Quant-iT PicoGreen® dsDNA Assay Kit. Error bars are s.d.




### B. Rolling Circle Amplification (RCA) from single-stranded M13(+) DNA

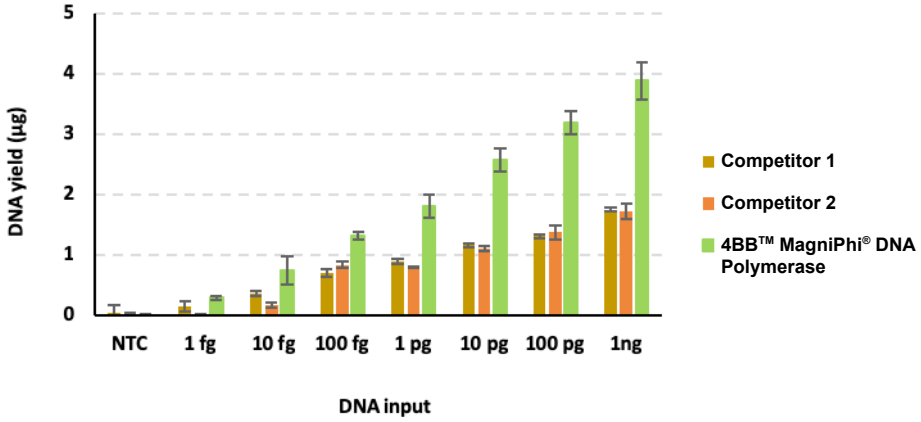
Different DNA doses ranging from 1fg to 1ng of single-stranded M13(+) DNA (GE Healthcare Lifescience Ref. 27-1546-01) were used as input in the amplification reactions. Input DNA was subjected to the workflow detailed above. Reaction mixtures were incubated for 3 hours at 30ºC and 4BB™ MagniPhi® DNA Polymerase was inactivated for 10 min at 65ºC, to avoid degradation of the amplification products. Amplified DNA was quantified using the Quant-iT PicoGreen® dsDNA Assay Kit. Error bars are s.d.



## BENCHMARKING

### A. Whole Genome Amplification (WGA) from high molecular weight human genomic DNA

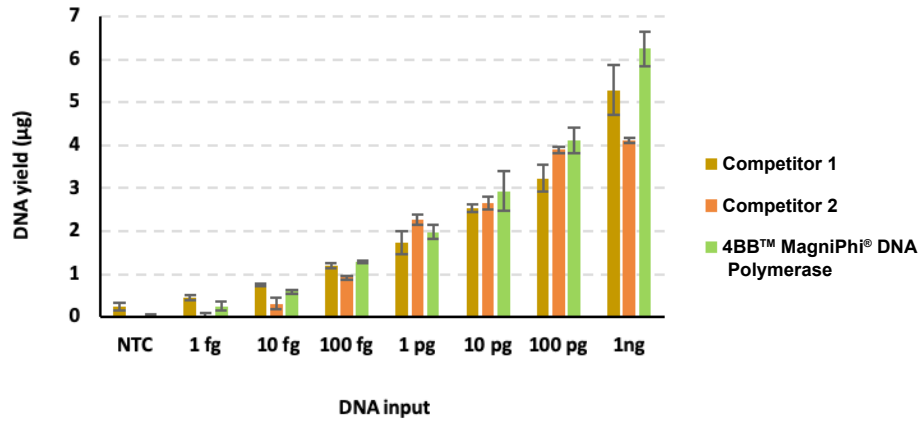
Different DNA doses ranging from 1 fg to 1 ng of human genomic DNA (Promega Ref. G147A) were amplified using Competitor 1, Competitor 2 and 4basebio 4BB™ MagniPhi® DNA Polymerase following the protocol described above. 100ng of each wild type Phi29 polymerase and 120ng of 4BB™ MagniPhi™ DNA Polymerase were used to test the same molar concentration. Reaction buffers from each provider were used in each case. Reaction mixtures were incubated for 3 hours at 30ºC and inactivated for 10 min at 65ºC, to avoid degradation of the amplification products. Amplified DNA was quantified using the Quant-iT PicoGreen® dsDNA Assay Kit. Error bars are s.d.




4BB™ MagniPhi® DNA Polymerase shows better sensitivity and higher yields than wild-type Phi29 DNA pol from different providers.

### B. Rolling Circle Amplification (RCA) from single-stranded M13(+) DNA

Different DNA doses ranging from 1fg to 1ng of single-stranded M13(+) DNA (GE Healthcare Lifescience Ref. 27-1546-01) were used as input in the amplification reactions. Input DNA was subjected to the workflow detailed above. Reaction mixtures were incubated for 3 hours at 30ºC and 4BB™ MagniPhi® DNA Polymerase was inactivated for 10 min at 65ºC, to avoid degradation of the amplification products. Amplified DNA was quantified using the Quant-iT PicoGreen® dsDNA Assay Kit. Error bars are s.d.

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