phi29 DNA Polymerase

Catalogue number: MB42301, 250 U

Description

phi29 DNA Polymerase is a highly processive non-thermostable polymerase displaying strong strand displacement activity, which allows highly efficient isothermal DNA amplification. phi29 DNA Polymerase is the replicative polymerase from the *Bacillus subtilis* phage phi29 and possesses a strong 3'→5' exonuclease (proofreading) activity. It acts preferentially on single-stranded DNA or RNA. phi29 DNA Polymerase is particular efficient for Whole Genome Amplification (WGA) through Multiple Displacement Amplification (MDA).

Storage conditions

phi29 DNA Polymerase should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme that will incorporate 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.

Enzyme concentration: 10 U/µL

Inactivation

phi29 DNA Polymerase is heat inactivated at 65 °C for 10 min.

System components and Reaction conditions

phi29 DNA Polymerase is provided with a dedicated highly optimized NZYTech reaction buffer and displays an optimum temperature of 30 °C. The presence of active reducing reagent in the reaction buffer is critical for this enzyme. Older buffer stocks or stocks that have been repeatedly frozen and thawed should be supplemented with 4 mM DTT to obtain maximal activity.

Standard protocol

The following standard protocol serves as a general guideline for Whole Genome Amplification (WGA) with phi29 DNA Polymerase. DNA samples need to be previously denatured and neutralized following standard protocols. Preferably the enzyme should be added last.

1. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA	10 – 50 ng
phi29 DNA Polymerase reaction buffer (10x)	5 µL
dNTPs	0.5 mM
Exo-resistant Random Hexamer Primers	5 µM
phi29 DNA Polymerase	1 µL (10 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 50 µL

2. Gently mix and pulse.
3. Incubate at 30 °C for 90 minutes.
4. Inactivate the enzyme for 10 min at 65 °C.
5. Proceed with downstream application, e.g. PCR (10-20 fold dilution with TE buffer, use 1-3 µL of diluted DNA) or DNA Labelling. Alternatively, store amplified DNA at -20 °C. Avoid repeated freeze-thaw-cycles

Quality Control Assays

Purity

phi29 DNA Polymerase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 10 U of phi29 DNA Polymerase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of phi29 DNA Polymerase for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

phi29 DNA Polymerase is assayed in a Multiple Displacement Amplification WGA protocol from human gDNA. Activity is measured by quantifying amplified DNA in a qPCR experiment of specific human genomic targets.

V2101

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases contamination	Pass
Functional assay	Pass

Approved by:

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