phi29 II DNA Polymerase

REF: EG25109-S/M

Storage Condition

Store at -20℃ for 2 years.

Components

Components	EG25109S	EG25109M
phi29 II DNA Polymerase (10 U/μl)	50 μl	5×50 μl
10× phi29 II Buffer	1 ml	2×1 ml

Description

phi29 II DNA Polymerase is engineered from the original phi29 DNA Polymerase with significantly improved performance. This enzyme is significantly improved over phi29 DNA polymerase in enhanced thermostability, amplification yield and sensitivity, while retaining all the benefits of the wild-type enzyme, with optimal amplification achieved at 42℃ .

Definition of Activity Unit

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

Applications

1. Rolling circle amplification (RCA)
2. Unbiased amplification of whole genome (WGA)
3. Multiple displacement amplification (MDA)
4. Single-cell genome amplification
5. Cell-free cloning of lethal DNA

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 19 μl reaction containing 200 ng of supercoiled plasmid and 10 U of phi29 II DNA Polymerase incubated for 4 hours at 37 ℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 19 μl reaction containing 15 ng of dsDNA fragments and 10 U of phi29 DNA Polymerase incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 U of phi29 DNA Polymerase incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

The residual amount of E.coli host cell DNA in this product is less than 1 copy/10 U.

Protocol

1. Prepare the following reaction system in an EP tube:

Reagent	Amount
Template[a]	1 μl
10× phi29 II Buffer[b]	2 μl
dNTP (10 mM)	2 μl
Random Primer (100 μM)[c]	2 μl
Pyrophosphatase, Inorganic (Yeast)[d] (0.1 U/μl)	1 μl
ddH_2O	Up to 19 μl

a. For genomic templates, the recommended dosage is 1 to 50 ng; for plasmid templates, the recommended dosage is 0.1 to 10 ng.
b. Crystals may form at the bottom of the tube after storage at -20℃ . These will dissolve upon thawing, and this does not affect performance.
c. For genomic templates, the recommended dosage is 1 to 50 ng; for plasmid templates, the recommended dosage is 0.1 to 10 ng.
d. Pyrophosphatase, Inorganic (Yeast) (REF: EG23109) is optional. Adding Pyrophosphatase, Inorganic (Yeast) can reduce the generation of by-products and increase the yield of the target fragment.

2. Incubate at 95℃ for 3 min before placing it on ice for 3 min, and add 1 μl of phi29 II DNA Polymerase (10 U/μl).

3. Incubate at 42℃ for 1~16 h，65℃ 10 min.

Notice

1. TelN Protelomerase activity is significantly inhibited in 10× phi29 II Buffer. If TelN Protelomerase digestion is required for phi29 II amplification products, please purify the products prior to the digestion reaction.

2. The product has extremely high sensitivity. Be careful to prevent template contamination.

3. The product is active within the temperature range of 30 to 42℃ , and the reaction temperature can be adjusted according to specific experimental requirements.

4. For research use only. Not for use in diagnostic procedures or other uses.

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