Check the product label for actual catalog number, lot and expiry date.

phi29 DNA Polymerase, 10 u/µl

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IDE0101	1000 u	1000 u – phi29 DNA Polymerase, 10 u/µl 0.5 ml – 10X phi29 Buffer	Enzyme storage buffer includes stabilizers and 50% glycerol. 10X phi29 Buffer contains 10 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, and other components.
IDE0105	5000 u	5 x 1000 u – phi29 DNA Polymerase, 10 u/µl 5 x 0.5 ml – 10X phi29 Buffer	

Storage *In the dark at -20°C.*

APPLICATIONS

- Isothermal DNA amplification for sequencing, cloning
- RCA – rolling cycle amplification
- MDA – multiple displacement amplification
- WGA - whole genome amplification
- Protein primed or RNA primed DNA amplification

BENEFITS

- High yield DNA amplification at constant 30°C temperature
- Robust polymerase with strong strand displacement activity
- High processivity, synthesis of over 70 kb long DNA strains
- High fidelity, low error rate for sequencing and cloning
- Classical enzyme/buffer formulation for known phi29 applications

PRODUCT DETAILS

Recombinant phi29 DNA Polymerase is a classical enzyme dedicated for use in common isothermal DNA amplification applications that are carried out at moderate temperature based on a strand displacement activity. The enzyme is supplied with an optimized high-performance buffer. The user has to add dNTPs, template and primers. The phi29 polymerase has strong strand displacement activity and efficient 5' - 3' polymerase activity working at about 4 - 35°C and synthesizing DNA from minor amounts to enormous yield up to visibly increased the viscosity of the reaction mixture. The enzyme has no 5' - 3' exonuclease activity, but has strong 3' - 5' exonuclease (proofreading) activity, and may degrade primers, therefore the use of 3' protected exo-resistant primers is widely recommended. The enzyme can be heat-inactivated, tolerates dUTP and produces blunt-ended DNA.

TECHNICAL CHARACTERISTICS

Most important technical characteristics of phi29 DNA Polymerase (based on abundantly available scientific literature):

- Strong strand displacement activity
- 5' - 3' polymerase activity on DNA templates (and on RNA templates)
- Strong 3' - 5' (proofreading) exonuclease activity degrades unprotected primers
- No 5' - 3' exonuclease activity
- Optimal reaction temperature is 30°C
- Working temperature range is 4 - 35°C, depends on application
- Optimal reaction time depends on application
- The enzyme is inactivated in 10 minutes at 65°C.
- Can extend both DNA and RNA primers
- Addition of pyrophosphatase may accelerate the reaction

The use of this product in certain applications, some buffer additives or certain protocols may be covered by patents. The user has to analyse all applicable Limited Use Label Licenses and may need licensing.

ENZYME USAGE GUIDELINES

- The usage guidelines are only very general. Follow the dedicated application protocol from the available literature sources, analyze all related applicable licenses and patents before the use of the enzyme.
- Take typical measures to prevent contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform denaturation/primer annealing by heating the reaction to 95°C and cooling it down fast. Only then add the phi29 polymerase to start the synthesis.
- Perform the reaction at 30°C. If needed, optimize the reaction temperature between 4 - 35°C for each template/primer system.
- Suggested reaction time is 60 - 360 minutes. For some low copy number targets overnight incubation might be required.
- Abundant amplification product may increase the viscosity of the solution, mix well and dilute if needed for downstream applications.

An example for enzyme and buffer amounts to prepare a 20 µl reaction:

10X phi29 Buffer	1 µl
100 µM Primers (for example, exo-resistant random hexamer, depends on application)	1 µl
Template DNA (variable, depends on application)	1 µl (from 1 to 10 ng)
PCR-grade Water	to 10 µl
✓ Mix gently, avoid bubbles. ✓ Place into the thermostat or qPCR instrument to anneal primers:	
Denaturation/primer annealing Cool down on ice or in the cycler	95°C – 3-5 min 4°C – 2-3 min
Add 10 mM dNTP Mix (#NUM0201)	1-2 µl
Add 10X phi29 Buffer (second portion)	1 µl
Add 50X qPCR-suited dye (optional for qPCR)	0.4 µl
Add PCR Water (#WAT0110)	to 19 µl
Add phi29 DNA Polymerase	1 µl

✓ Mix and incubate for the DNA synthesis at 30°C for 1 - 6 hours.
✓ Enzyme can be inactivated in 10 minutes at 65°C.
✓ Store reactions for short time at +4, for long time at -20°C.

IN VITRO RESEARCH USE ONLY