ArciTect™ High-Fidelity DNA Polymerase Kit

Polymerase, buffers, and dNTPs for high-fidelity PCR amplification

Catalog # 76026 1 Kit 500 Reactions

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Product Description

ArciTect™ High-Fidelity DNA Polymerase Kit is optimized for robust, high-fidelity DNA amplification (> 50X higher than *Taq*). It may be used in applications requiring ultra-low error rates, such as detection of genome editing with ArciTect™ T7 endonuclease I (refer to the Technical Bulletin: Evaluation of Genome Editing, Document #27126), as well as sequencing, cloning/subcloning, synthetic biology, and SNP analysis. ArciTect™ High-Fidelity DNA Polymerase is a fusion protein with a double-stranded DNA-binding domain and a Pyrococcus-like proofreading polymerase domain.

ArciTect™ High-Fidelity DNA Polymerase Kit includes ArciTect™ High-Fidelity DNA Polymerase, dNTP Mix (10 mM; containing dATP, dCTP, dGTP, and dTTP sodium salts), and two buffers: ArciTect™ High-Fidelity Buffer (for standard high-fidelity reactions) and ArciTect™ High GC Content Buffer (for difficult-to-amplify templates that are rich in G and C bases). Reactions using ArciTect™ High GC Content Buffer have increased sensitivity with a slightly higher error rate. All components are animal component-free.

ArciTect™ High-Fidelity DNA Polymerase exhibits a 50- to 60-fold lower error rate than standard *Taq* polymerase using the lacI mutagenesis assay. It has an extension rate of 67 nucleotides per second and can successfully amplify long targets (up to 5 kb human genomic DNA and 8 kb of lambda DNA). This enzyme generates blunt-end products.

Product Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
ArciTect™ High-Fidelity DNA Polymerase Kit	76026	1 Kit - 500 Reactions	• dNTP Mix (10 mM), 1 mL • ArciTect™ High-Fidelity DNA Polymerase, 250 µL • ArciTect™ High-Fidelity Buffer, 4 x 1.5 mL • ArciTect™ High GC Content Buffer, 4 x 1.5 mL

Component Storage and Stability

The following components are sold as a complete kit (see Product Information) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
dNTP Mix (10 mM)	76027	1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
ArciTect™ High-Fidelity DNA Polymerase	76029	250 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
ArciTect™ High-Fidelity Buffer	76030	1.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
ArciTect™ High GC Content Buffer	76031	1.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

Specifications for ArciTect™ High-Fidelity DNA Polymerase

Formulation: 20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, stabilizer, 50% glycerol, pH 7.4 at 25°C

Molecular Weight: 97.7 kDa

Source: E. coli

Activity: 2 U/µL; 1 Unit is defined as the amount of enzyme required to incorporate 10 nmol of dNTPs into acid-insoluble form at 74°C in 30 minutes.

Extension Rate: 67 kb/second

3' to 5' Exonuclease Activity: (proofreading) Yes

5' to 3' Exonuclease Activity: (nick translation) No

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Total DNA purification kit	e.g. QIAGEN 69504
Microcentrifuge tubes	e.g. 38089
PCR tubes or strips	e.g. 38091 or 38094
Forward and reverse primers	---
DNA template	---
Nuclease-Free Water	79001

Directions for Use

1. Purify DNA sample using a total DNA purification kit. Store on ice.
2. Thaw either ArciTect™ High-Fidelity Buffer or ArciTect™ High GC Content Buffer at room temperature (15 - 25°C).

   NOTE: Use ArciTect™ High-Fidelity Buffer for standard high-fidelity amplifications; use ArciTect™ High GC Buffer for GC-rich/difficult templates.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. Once aliquots are thawed, do not re-freeze.
3. Centrifuge polymerase briefly to collect material at bottom of tube. Store on ice until use.

   NOTE: ArciTect™ High-Fidelity DNA Polymerase may appear cloudy due to the presence of stabilizer. Product performance will not be affected.
4. To prepare the Reagent Mix, combine components in a microcentrifuge tube on ice as indicated in Table 1. Indicated volumes are for preparing 50 µL of Reagent Mix. If preparing other volumes, adjust accordingly.

Table 1. Preparation of Reagent Mix

COMPONENT	VOLUME (µL)	FINAL AMOUNT/CONCENTRATION
ArciTect™ High-Fidelity Buffer OR ArciTect™ High GC Content Buffer	10	1X $MgCl_2$: 1.5 mM*
dNTP Mix (10 mM)	1	200 µM each
10 µM Forward primer	1**	0.2 µM
10 µM Reverse primer	1**	0.2 µM
DNA template	Variable	50 - 250 ng†
ArciTect™ High-Fidelity DNA Polymerase	0.5	1 U††
Nuclease-free water	Variable	Bring solution to total volume of 50 µL

*If desired, increase [$MgCl_2$] in 0.2 µM increments, up to 3.0 mM; [$MgCl_2$] > 3.0 mM may reduce fidelity.
**Use up to 5 µL (1 µM final concentration); 1 µL (0.2 µM) is recommended for most applications.
†For low-complexity genomes (e.g. plasmid, virus, or bacterial artificial chromosome), 1 pg - 10 ng is recommended.
††For long targets (> 1 kb), difficult templates, or for higher yield, use up to 2 U polymerase.

5. Briefly centrifuge the Reagent Mix.
6. Perform PCR in a thermocycler using the conditions indicated in Table 2. For PCR troubleshooting, see Notes and Tips.

Table 2. Recommended PCR Cycling Conditions

STEP	TEMPERATURE	TIME
Initial denaturation	98°C	30 seconds *to 3 minutes**
Denaturation, annealing, extension for 15 - 35 cycles	98°C	5 - 10 seconds
	Varies**	10 - 30 seconds
	72°C	15 - 30 seconds per kilobyte of DNA
Final extension	72°C	5 - 10 minutes
Hold	4°C	Up to 24 hours

*For difficult templates, initial denaturation can be extended up to 3 minutes.
**For primers over 20 nucleotides long, the annealing temperature should be ~3°C higher than the lowest melting temperature (Tm). For primers shorter than 20 nucleotides, the annealing temperature should equal the lowest Tm. If the Tm of the primer pairs is ≥ 72°C, the annealing and extension steps can be combined into a two-step cycling program.

Notes and Tips

- To improve PCR yield, increase the extension time and/or template concentration. PCR enhancers can be used (e.g. betaine, DTT, BSA, or DMSO) to help with complex targets.
- DMSO may be used to reduce secondary structure of GC-rich templates. DMSO is usually used at a 3% (v/v) final concentration, but up to 9% has been used to improve success rate on difficult PCR templates. If using DMSO, a lowered annealing temperature is recommended.

Related Products

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