

ArciTect™ High-Fidelity DNA Polymerase Kit



Scientists Helping Scientists™ | WWW.STEMCELL.COM

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Catalog #76026 1 Kit 500 Reactions

Product Description

ArciTect™ High-Fidelity DNA Polymerase Kit is optimized for robust, high-fidelity DNA amplification (> 50X higher than *Taq* polymerase). It may be used in applications requiring ultra-low error rates, such as detection of genome editing with ArciTect™ T7 Endonuclease I Kit (Catalog #76021; 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 of this kit are animal component-free.

ArciTect™ High-Fidelity DNA Polymerase exhibits a 50- to 60-fold lower error rate than standard *Taq* 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 of 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	<ul style="list-style-type: none">dNTP Mix, 1 mLArciTect™ High-Fidelity DNA Polymerase, 250 µLArciTect™ High-Fidelity Buffer, 6 x 1.5 mLArciTect™ High GC Content Buffer, 3 x 1.5 mL

Component Storage and Stability

The following components are sold as a complete kit (see Product Information).

dNTP Mix (Catalog #76027) is also available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
dNTP Mix	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:	<i>E. coli</i>
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:	Yes (proofreading)
5' to 3' Exonuclease Activity:	No (nick translation)

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Genomic DNA Purification Kit	79020
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

- Purify DNA sample using the Genomic DNA Purification Kit. Store on ice.
- 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. After thawing the aliquots, use immediately; do not re-freeze.
- 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.
- 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	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 [MgCl₂] in 0.2 µM increments, up to 3.0 mM; [MgCl₂] > 3 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.

- Briefly centrifuge the Reagent Mix.
- 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 (T_m). For primers shorter than 20 nucleotides, the annealing temperature should equal the lowest T_m. If the T_m 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

For related products, including other genome editing tools, specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2023 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and ArciTect are trademarks of STEMCELL Technologies Canada Inc. All other trademarks are the property of their respective holders. Sale of this product is covered under at least US Patent Nos. 6,627,424, 7,541,170, 7,560,260, and corresponding patents in other countries or other patents pending and all applicable STEMCELL Terms and Conditions of Sale. This product can be used solely for DNA sequencing, DNA micro-array and conventional PCR applications in the life science research field, but not real-time PCR or digital PCR. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.