AccuStart™ Taq DNA Polymerase HiFi

Concentration: 5 units/µL

Cat. No. 95085-250 Size: 250 units **Store at -20°C**
95085-01K 1000 units
95085-05K 5000 units

Description

AccuStart Taq DNA Polymerase HiFi is an enzyme mixture of recombinant Taq DNA polymerase preparation, a thermal stable DNA polymerase with 3'→5' exonuclease activity, and monoclonal antibodies that bind to the polymerase and keep it inactive before PCR thermal cycling (1). Upon heat activation (1 minute at 94°C), the antibodies denature irreversibly, releasing fully active DNA polymerase. Non-specific extension of primers at low temperatures is a common cause of artifacts and poor sensitivity in PCR. The AccuStart automatic hot-start enables specific and efficient primer extension in the PCR process with the added convenience of room temperature reaction assembly. This enzyme mixture and optimized HiFi PCR buffer improves the fidelity of DNA synthesis approximately 6-fold higher than Taq DNA polymerase alone and enables amplification of DNA fragments up to 20-kb long (2). AccuStart Taq DNA Polymerase HiFi is a robust alternative PCR enzyme for both routine PCR applications as well as amplification of problematic templates.

Components

AccuStart Taq DNA polymerase HiFi	5 units/µL in 50% glycerol, 20 mM Tris-HCl, 40 mM NaCl, 0.1 mM EDTA, and stabilizers.
HiFi PCR Buffer (10X)	600 mM Tris-SO_4 (pH 8.9), 180 mM $(NH_4)_2SO_4$
50 mM magnesium sulfate	50 mM $MgSO_4$

Storage and Stability

AccuStart Taq DNA polymerase HiFi is stable for 2 years when stored in a constant temperature freezer at -20°C.

General PCR protocol

The following procedure is presented as general guideline for using AccuStart Taq DNA Polymerase HiFi in any PCR procedure. Cycling conditions, concentration of, primers, $MgSO_4$, and dNTPs, and the amount of AccuStart Taq DNA Polymerase HiFi may need to be optimized. Preparation of a master mix cocktail that contains all components except DNA template when performing multiple PCRs with the same primer set. Reaction volume may be scaled to suit individual needs.

Since as little as one molecule of DNA template can initiate the PCR process, it is important to take appropriate precautions to avoid contamination of reagents with DNA template and cross-sample contamination. Assemble reactions (without template) in a DNA-free area using dedicated pipettors and aerosol-resistant barrier tips. Add DNA template to reactions as the final step. Change gloves frequently. Ideally, the PCR workflow should be segregated into separate areas for reaction assembly, processing/addition of DNA template(s), and analysis of PCR products.

Reaction Assembly

Add the following components to a thin-walled PCR tube:

Component	Volume for 50-µL rxn.	Final Concentration
Nuclease-free water	variable	
HiFi PCR Buffer (10X)	5 µL	1x
50 mM magnesium sulfate	2 µL	2 mM
10 mM dNTP Mix	1 µL	200 µM each dNTP
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
AccuStart Taq DNA Polymerase HiFi	0.2 µL	1 unit
DNA Template	5 – 10 µL	variable
Final Volume (µL)	50 µL	

Temperature Cycling Protocol

Incubate the completed reaction mix in thermal cycler as follows:

Initial denaturation:	94ºC, 1 min
PCR cycling (20 – 40 cycles:)	94ºC, 15 to 20 s 55 – 65ºC, 30s 68ºC, 1 min per kb of product length
Hold	4ºC until processed for analysis

Full activation of AccuStart Taq DNA Polymerase HiFi occurs within 30 seconds at 94ºC; however, complete denaturation of double-stranded DNA template is required to initiate the PCR process. Consequently, the initial denaturation time may require optimization depending on the nature and properties of a given target sequence. A 1-minute initial denaturation is sufficient for amplification of most templates. Amplification supercoiled DNA templates may require a longer initial denaturation time to fully denature the template prior to PCR cycling. Initial denature times should be kept to a minimum when amplifying long fragments to avoid temperature induced DNA damage (deamination, depurination, and strand cleavage).

Quality Control

Kit components are free of contaminating DNase and RNase. AccuStart Taq DNA Polymerase HiFi is functionally tested for amplification of a 20-kb fragment from human genomic DNA. Inhibition of polymerase activity by the AccuStart anti-Taq monoclonal antibodies is tested in an activity assay that measures polymerase inhibition relative to an uninhibited control.

Unit definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74°C.

References

1 Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) BioTechnology, 12

2 Barnes, W.M. (1994) Proc. Natl. Acad. Sci. USA 91, 2216.

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