AccuStart™ Taq DNA Polymerase

Concentration: 5 units/µL

Cat. No.		Size:	
	95061-250		250 units
	95061-01K		1000 units
	95061-05K		5000 units

Store at -20°C

Description

AccuStart Taq DNA Polymerase is a recombinant Taq DNA polymerase preparation which contains monoclonal antibodies that bind to the polymerase and keep it inactive before PCR thermal cycling. Upon heat activation (1 minute at 94ºC), the antibodies denature irreversibly, releasing fully active Taq 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.

Activated AccuStart Taq DNA polymerase possesses 5'→3' DNA polymerase activity and a double-strand specific 5'→3' exonuclease. The polymerase does not have 3'-exonuclease activity and is free of any contaminating endo or exonuclease activities. 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.

Components

AccuStart Taq DNA polymerase	5 units/µL in 50% glycerol, 20 mM Tris-HCl, 40 mM NaCl, 0.1 mM EDTA, and stabilizers.
10X PCR Buffer	0.2M Tris-HCl (pH 8.4), 0.5 M KCl
50 mM magnesium chloride	50 mM $MgCl_2$

Storage and Stability

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

General PCR protocol

The following procedure is presented as general guideline for using AccuStart Taq DNA Polymerase in any PCR procedure. Cycling conditions, concentration of, primers, $MgCl_2$, and dNTPs, and the amount of AccuStart Taq DNA polymerase may need to be optimized. AccuStart Taq DNA polymerase is ideally suited for the 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	
10X PCR Buffer	5 µL	1x
50 mM magnesium chloride	1.5 µL	1.5 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	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 to 3 min
PCR cycling (20 – 40 cycles:)	94ºC, 15 to 30 s 55 – 65ºC, 30s 68 – 72ºC, 1 min per kb of product length
Hold	4ºC until processed for analysis

Full activation of AccuStart Taq DNA polymerase 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 cDNA and short linear dsDNA fragments. Amplification of genomic DNA or supercoiled DNA templates may benefit from a 3 to 10 minute incubation to fragment the template and 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 is functionally tested for amplification of a 4-kb fragment from a single-copy gene in 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. AccuStart Taq DNA polymerase is tested to be free of any possible mouse genomic DNA.

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