# AccuStart™ II Taq DNA Polymerase

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| Cat No. | 95141-250 | Size: | 250 units | **Store at -25ºC to - 15°C** |
| | 95141-01K | | 1000 units | |
| | 95141-05K | | 5000 units | |

## Description

AccuStart II Taq DNA Polymerase is a high purity, recombinant Taq DNA polymerase preparation with high avidity monoclonal antibodies that bind the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (1 minute at 94ºC), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly. Non-specific extension of primers at low temperatures is a common cause of artifacts and poor sensitivity in PCR. The AccuStart II automatic hot-start enables specific and efficient primer extension in the PCR process with the added convenience of room temperature reaction assembly. The included 10X PCR Buffer II is a new optimized buffer that provides higher product yield, improved specificity, and enhanced multiplexing capability.

Activated AccuStart II 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. AccuStart II Taq DNA polymerase contains extremely low levels of residual host, *E. coli* genomic DNA.

## Components

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| AccuStart II 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 II | Optimized 10X-concentrated buffer |
| 50 mM magnesium chloride | 50 mM $MgCl_2$ |

## Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

## General PCR protocol

The following procedure is presented as general guideline for using AccuStart II Taq DNA Polymerase in any PCR procedure. Cycling conditions, concentration of, primers, $MgCl_2$, and dNTPs, and the amount of AccuStart II Taq DNA polymerase may need to be optimized. AccuStart II 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 II | 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 II 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:

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| Initial denaturation: | 94ºC, 1 to 3 min |
| PCR cycling (20 – 40 cycles:) | 94ºC, 15 to 30 s<br>55 – 65ºC, 30s<br>68 – 72ºC, 1 min per kb of product length |
| Hold | 4ºC until processed for analysis |

Full activation of AccuStart II 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 II 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 II anti-Taq monoclonal antibodies is tested in an activity assay that measures polymerase inhibition relative to an uninhibited control. AccuStart II Taq DNA polymerase is tested to be free of any possible mouse genomic DNA and to contain less than 1 copy of residual *E. coli* genomic DNA per unit of enzyme.

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