



AccuStart™ II PCR ToughMix® (2X)

Cat. No.	95142-100	Size:	100 x 25 µL reactions (1x 1.25 mL)
	95142-800		800 x 25 µL reactions (8 x 1.25 mL)
	95142-04K		4000 x 25 µL reactions (1 x 50 mL)

Store at -20°C

Description

AccuStart II PCR ToughMix is a 2X concentrated ready-to-use reaction cocktail for PCR amplification of DNA templates that overcomes many known inhibitors of PCR often present in crude samples extracted from environmental specimens, plant tissues, or animal tissues. It contains all components, except primers and template. A key component of AccuStart PCR ToughMix is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. These antibodies bind the polymerase and keep it inactive prior to the initial PCR denaturation step. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly. Similar to Taq DNA polymerase, the activated polymerase in AccuStart II PCR ToughMix 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. PCR products generally contain non-templated dA additions and can be cloned using vectors that have a single 3'-overhanging thymine residue on each end.

GelTrack® Loading Dye is a mixture of blue and yellow electrophoresis-tracking dyes that migrate at approximately 4kb and 50 bp. This optional component simplifies post PCR analysis, allowing direct loading of PCR product on agarose gels following amplification. The GelTrack Loading Dye solution is not included with the sample kit.

Components

AccuStart II GelTrack PCR ToughMix (2X)	2X mix containing optimized concentrations of MgCl ₂ , dNTPs, reaction buffer, hot-start DNA polymerase, stabilizers and gel loading dyes.
GelTrack Loading Dye (50X)	50X concentrated mixture of RT-PCR compatible, blue and yellow electrophoresis-tracking dyes.

Storage and Stability

AccuStart II PCR ToughMix is stable for 1 year when stored in a constant temperature freezer at -20°C. After thawing, mix thoroughly before using. For convenience, it may be stored unfrozen at +2 to +8°C for up to 6 months. Repeated freezing and thawing does not affect product performance.

Reaction Assembly

Component	Volume for 25-µL rxn.	Final Concentration
AccuStart II PCR ToughMix (2X)	12.5 µL	1X
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
GelTrack Loading Dye (50X)	0.5 µL	1X
Nuclease-free water	variable	
DNA Template	<u>5 – 10 µL</u>	Variable
Final Volume (µL)	25 µL	

Note: For smaller or larger reaction volumes (10 to 50 µL), scale all components proportionally.

Reaction Protocol

Incubate the completed reaction mix in thermal cycler as follows:

Initial denaturation:	94°C, 1 to 3 min
PCR cycling (20 – 40 cycles:)	94°C, 10 to 30 s 55 – 65°C, 15 to 30s 68 – 72°C, 1 min per kb of product length
Hold	4°C until processed for analysis

Full activation of the DNA polymerase occurs within 30 seconds at 94°C. Complete denaturation of dsDNA target is important for efficient PCR amplification and may require different initial denaturation times depending on the properties of a given target sequence. Optimal annealing temperature may need to be empirically determined for your primers.

Quality Control

Kit components are free of contaminating DNase and RNase. AccuStart II PCR ToughMix is functionally tested for amplification of a 4-kb fragment from a single-copy gene in human genomic DNA.

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