

AccuStart™ II PCR ToughMix™

Tough PCR for Tough Samples

AccuStart II PCR ToughMix is a 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.



Quanta
BIOSCIENCES™

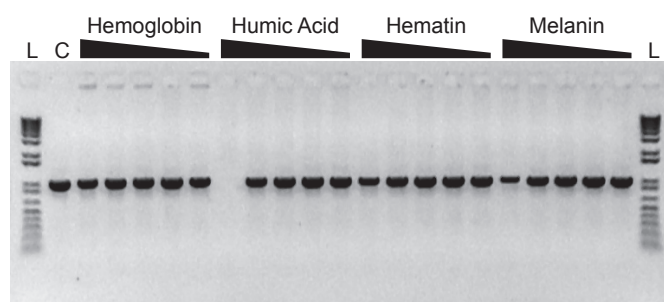
FEATURES AND BENEFITS

- Robust mix designed to easily overcome PCR inhibitors
- Stringent antibody hot start
- Optional Gel Track Loading Dye included
- Convenient reaction assembly at room temperature

AccuStart II PCR ToughMix

AccuStart II PCR ToughMix is a 2X concentrated ready-to-use reaction cocktail for the PCR amplification of DNA templates in the presence of inhibitors. 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.

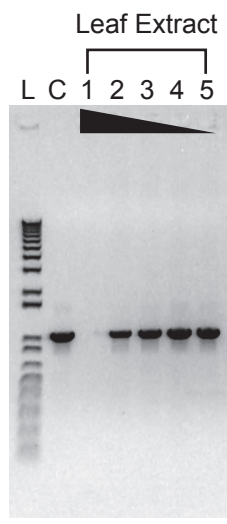


30 cycle PCR; 1 x 10⁴ copies TcR DNA (1052-bp amplicon)

Inhibitor Resistance of AccuStart II PCR ToughMix

A 1-kb fragment from 1e4 copies of the Tetracyclin resistance gene was amplified in 20- μ L reaction volumes according to the recommended protocol. Reactions were challenged with varying concentrations of different PCR inhibitors as summarized below. Following a 3 min activation at 94°C; PCR was for 30 cycles of: 94°C, 15s; 60°C, 20s; 72°C, 1 min. 1/5th of each reaction was analyzed on a 0.1% agarose, 0.5X TBE gel containing 0.25 mg/mL ethidium bromide.

Hemoglobin: 316 ng/ μ L, 100 ng/ μ L, 31.6 ng/ μ L, 10 ng/ μ L, 3.16 ng/ μ L
Humic Acid: 31.6 ng/ μ L, 10 ng/ μ L, 3.16 ng/ μ L, 1 ng/ μ L, 0.316 ng/ μ L
Hematin: 100 μ M, 31.6 μ M, 10 μ M, 3.16 μ M, 1 μ M
Melanin: 10 ng/ μ L, 3.16 ng/ μ L, 1 ng/ μ L, 0.316 ng/ μ L, 0.1 ng/ μ L
C: control reactions without inhibitor
L: 1 Kb Plus DNA Ladder (Invitrogen)



30 cycle PCR; 1 x 10⁴ copies Tcr DNA (1052-bp amplicon)

Inhibitor Resistance of AccuStart II PCR ToughMix: PCR in the presence of polyphenol spike

Varying amounts of a polyphenol-rich plant extract (0.2, 0.06, 0.02, 0.006, or 0.002 uL) were added to 25-uL PCRs containing 10,000 copies of a control template. Amplification was carried out for 30 cycles of: 94°C, 15s; 60°C, 20s; 72°C, 1 min. 1/5th of each reaction was analyzed on a 0.1% agarose, 0.5X TBE gel containing 0.25 mg/mL ethidium bromide. As little as 0.002 uL of the crude plant lysate inhibited control reactions with a conventional PCR master mix (data not shown).

COMPONENTS

- AccuStart II GelTrack PCR ToughMix (2X) 2X mix containing optimized concentrations of MgCl₂, 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.

ORDERING INFORMATION

PRODUCT	Quanta Cat. No.	Pack Size (20uL Reactions)
AccuStart II PCR ToughMix	95142-800	800 x 25 µL reactions (8 x 1.25 mL)
	95142-04K	4000 x 25 µL reactions (1 x 50 mL)