

AccuStart II PCR ToughMix[®]

Robust, reliable PCR assay performance with challenging sample materials or impure templates

FEATURES AND BENEFITS:

- Stabilized 2x PCR SuperMix enables convenient room-temperature setup and is unaffected by repetitive freeze-thaw
- High-yielding, ultrapure modified Taq DNA polymerase delivers robust, reliable duplex assay performance
- Stringent, ultrapure antibody hotstart ensures sensitive and specific target amplification
- Separate electrophoretic mobility dye reduces risk of post-PCR cross contamination with gel electrophoresis

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. The only user supplied components are DNA template and molecular grade water.

A key component to AccuStart II 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 with gel electrophoresis and eliminates potential for cross contamination by enabling direct transfer of PCR products to the gel sample wells.

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

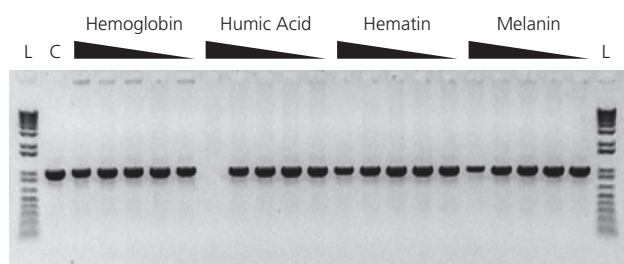


Figure 1 Inhibitor Resistance of AccuStart II PCR ToughMix. A 1-kb fragment from 1 x 10⁴ copies of the Tetracyclin resistance gene was amplified in 20 µl reaction volumes according to the recommended protocol. Reactions were challenged with varying concentrations of different PCR inhibitors as summarized below. Following a 3 min activation at 94°C; PCR was for 30 cycles of: 94°C, 15 s; 60°C, 20 s; 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/µl, 100 ng/µl, 31.6 ng/µl, 10 ng/µl, 3.16 ng/µl
 Humic Acid: 31.6 ng/µl, 10 ng/µl, 3.16 ng/µl, 1 ng/µl, 0.316 ng/µl
 Hematin: 100 µM, 31.6 µM, 10 µM, 3.16 µM, 1 µM
 Melanin: 10 ng/µl, 3.16 ng/µl, 1 ng/µl, 0.316 ng/µl, 0.1 ng/µl
 C: control reactions without inhibitor; L: 1 kb plus DNA Ladder (Invitrogen)

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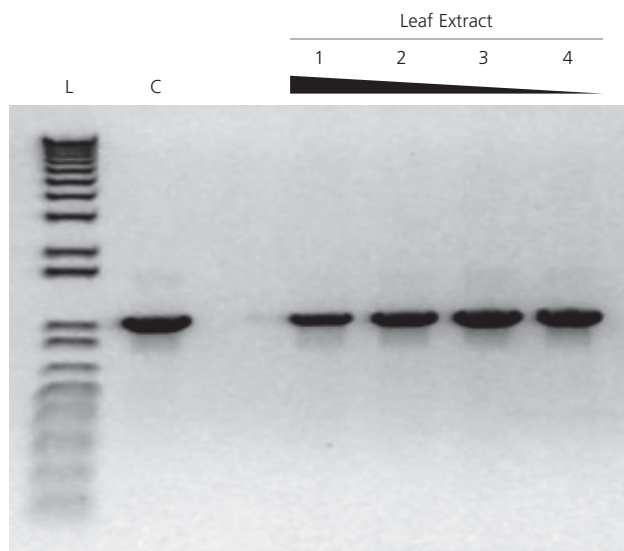


Figure 2 Inhibitor Resistance of AccuStart II PCR ToughMix: PCR in the Presence of Polyphenol Spike. Varying amounts of a polyphenol-rich plant extract (0.2, 0.06, 0.02, 0.006, or 0.002 μl) were added to 25 μl PCRs containing 10,000 copies of a control template. Amplification was carried out for 30 cycles of: 94°C, 15 s; 60°C, 20 s; 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 μl of the crude plant lysate inhibited control reactions with a conventional PCR master mix (data not shown).

ORDER INFO

Product Name

AccuStart II PCR ToughMix - 100 R
 AccuStart II PCR ToughMix - 800 R
 AccuStart II PCR ToughMix - 4000 R

Quantabio Catalog Number

95142-100
 95142-800
 95142-04K

Size

100 x 25 μl rxns (1 x 1.25 ml)
 800 x 25 μl rxns (8 x 1.25 ml)
 4000 x 25 μl rxns (1 x 50 ml)

Trademarks: ToughMix® is a registered trademark of Quantabio, LLC.

Quantabio products are intended for molecular biology applications. The products are not intended for the diagnosis, prevention or treatment of a disease.

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