

# AccuStart™ II PCR SuperMix (2X)

Cat No. 95137-100 Size: 100 x 25 µL reactions (1 x 1.25 mL) Store at -25°C to - 15°C

95137-500 500 x 25 µL reactions (5 x 1.25 mL) 95137-04K 4000 x 25 µL reactions (1 x 50 mL)

#### Description

AccuStart II PCR SuperMix is a 2X concentrated, ready-to-use reaction cocktail for routine PCR amplification of DNA fragments up to 4 kb followed by analysis on agarose gels. It contains all components, except primers and template. AccuStart II PCR SuperMix simplifies reaction assembly, improves assay reproducibility, and reduces the risk of contamination. AccuStart Taq DNA polymerase in the master mix is inactivated with monoclonal antibodies that bind the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (1 minute at 94°C), 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.

## Components

AccuStart II PCR SuperMix (2X) 2X mix containing optimized concentrations of MgCl<sub>2</sub>, dNTPs, reaction buffer, AccuStart Taq DNA

Polymerase, AccuStart Taq antibodies and stabilizers.

### Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.

Repeated freezing and thawing does not impair product performance.

For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

#### Reaction Assembly

| Component                      | Volume for 25-µL rxn. | Final Concentration |
|--------------------------------|-----------------------|---------------------|
| AccuStart II PCR SuperMix (2X) | 12.5 μL               | 1x                  |
| Forward primer                 | variable              | 100 – 500 nM        |
| Reverse primer                 | variable              | 100 – 500 nM        |
| 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:  $94^{\circ}$ C, 1 to 3 min PCR cycling (20 – 40 cycles:)  $94^{\circ}$ C, 10 to 30 s  $55 - 65^{\circ}$ C, 15 to 30s

00 - 00 C, 10 to 505

68 – 72°C, 1 min per kb of product length

Hold 4°C until processed for analysis

Full activation of Taq 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.

#### **Quality Control**

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

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