

| Product Information                             |                |
|---|----------------|
| <b>AccuStart™ Genotyping ToughMix®<br/>ROX®</b> |                |
| Part Number                                     | 95116-05K      |
| Number of Reactions                             | 5000 reactions |
| Reaction Size                                   | 20µL           |
| Storage Temperature                             | -25°C to -15°C |
| Lot Number                                      | 66194516       |
| Reference Number                                | 111621         |
| Expiration Date                                 | 08/30/2024     |

**Product Description:**

AccuStart Genotyping ToughMix ROX 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 is a versatile and robust real-time qPCR reagent that provides maximum sensitivity and PCR efficiency with a variety of fluorogenic probe chemistries, including TaqMan hydrolysis probes. The only user-supplied components are primers, probe(s), and DNA template. Inert AccuVue plate loading dye is compatible with either white or clear PCR plates and helps to minimize pipette error and provides visual confirmation of thorough mixing.

This proprietary formulation has been rigorously optimized to destabilize probes that differ by only a single base-pair substitution to provide precise allelic discrimination for genotyping single nucleotide polymorphisms (SNPs) with either hydrolysis (TaqMan) or FRET-based fluorescent probes.

**Component Part Numbers:**

**84205: AccuStart Genotyping ToughMix ROX, 50.0mL**

| Product Specifications |                                      |                                      |       |       |
|------------------------|--------------------------------------|--------------------------------------|-------|-------|
| 95116                  |                                      |                                      |       |       |
| Assay                  | RT-qPCR Plasmid DNA Functional Assay | RT-qPCR genomic DNA Functional Assay | DNase | RNase |
| Result                 | Pass                                 | Pass                                 | Pass  | Pass  |

**Quality Control Analysis and Specifications:**

**Nuclease Assay:**

**DNase:** DNase activity must be below the detectable limits of 100 pg DNase I equivalent as assayed using a fluorogenic substrate following a 1 hour incubation at 37°C with each kit component at 1X concentration.

**RNase:** RNase activity must be below the detectable limits of 1 pg RNase A equivalent as assayed using a fluorogenic substrate following a 1 hour incubation at 37°C with each kit component at 1X concentration.

**RT-qPCR Plasmid DNA Functional Assay:** Fast-cycling Real-time PCR detection of log-fold serial dilutions of a control DNA from 10 to  $1 \times 10^7$  copies. Linear regression analysis of cycle threshold versus log input quantity must give a slope of between -3.20 and -3.65 and coefficient of determination ( $R^2$ )  $\geq 0.990$ .

**RT-qPCR genomic DNA Functional Assay:** Real-time PCR detection of single-copy gene in human genomic DNA using activation step of 10 minutes at 95°C. Linear regression analysis of cycle threshold versus log input quantity for a log-fold serial dilutions of human genomic DNA from 10 to  $1 \times 10^5$  copies must give a slope of between -3.20 and -3.65 and coefficient of determination ( $R^2$ )  $\geq 0.990$  with accurate two-fold discrimination of 500, 1000, and 2000 copies.

**Limitations of Use**

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