AccuStart™ Genotyping ToughMix™, ROX™

Cat No. 95116-012 Size: 1250 x 20-µL reactions (10 x 1.25 mL)
95116-05K 5000 x 20-µL reactions (1 x 50 mL)

Store at -25ºC to - 15°C protected from light

Description

AccuStart Genotyping ToughMix, ROX is a 2X concentrated ready-to-use reaction cocktail for PCR amplification of DNA templates that overcomes inhibitors often present in crude samples extracted from environmental specimens, plant tissues, or animal tissues. It is a versatile and robust PCR reagent that provides highly specific and efficient amplification for genotyping sequence variants including allelic discrimination of single nucleotide polymorphisms (SNPs) using a variety of fluorogenic probe chemistries, including TaqMan® hydrolysis probes. AccuStart Genotyping ToughMix, ROX contains all required reaction components, except primers, probes, and DNA template. The light blue color of the AccuVue™ tracer dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting or mixing errors. It does not interfere with reporter dye fluorescence or affect the stability of the product.

A key component of AccuStart Genotyping ToughMix, ROX is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This proprietary polymerase mix is highly resistant to PCR inhibitors and provides an extremely stringent automatic hot-start allowing reaction assembly, and temporary storage, at room temperature prior to PCR amplification. AccuStart Genotyping ToughMix, ROX is compatible with both fast and conventional PCR cycling protocols.

Instrument Compatibility

Different real-time PCR systems or allelic discrimination analysis methods employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is important to match the appropriate reference dye to each specific optical detection system. AccuStart Genotyping ToughMix, ROX contains an optimal concentration of a stabilized carboxy-X-rhodamine compound (ROX™) for instruments that use an excitation wavelength of ~490 nm and 605 to 610 nm emission channel for the reference signal. Please consult our Product Finder selection tool at www.quantabio.com to find the correct product for your real-time PCR system.

Components

AccuStart Genotyping ToughMix, ROX (2X):	2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, ROX reference dye, AccuVue blue qPCR dye, and stabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt.
After thawing, mix thoroughly before using. Repeated freezing and thawing does not affect PCR performance.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for Allelic Discrimination PCR:

- The design of highly specific primers and probes is a critical parameter for successful genotyping using fluorogenic probes with PCR. The use of computer aided primer design programs is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, the primer pair, and primer/probe combinations. For best results, amplicon size should be limited to 65 - 200 bp. Optimal results may require titration of primer concentration between 100 and 900 nM. A final concentration of 300 - 400 nM each primer and 100 to 250 nM probe is effective for most applications. Increasing the concentration of the primer that initiates synthesis of the target strand that is complementary to the probe can improve fluorescent signal for some primer/probe systems.
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except sample template (genomic DNA or cDNA) and dispense equal aliquots into each reaction tube. Add the DNA template to each reaction as the final step. Addition of samples as 2 to 5-µL volumes will improve assay precision.
- ***Sample Template***: AccuStart Genotyping ToughMix, ROX can be used with purified genomic DNA or sample lysates. Use 1 to 10 ng of gDNA per reaction well. The optimal amount of lysate will vary for different sample types and lysate procedures. Empirical optimization is often required to determine the optimal dilution range. For rapid sample preparation from animal tissue specimens, we recommend our Extracta™ DNA Prep for PCR – Tissue (Cat. Nos. 95091-025 or 95091-250).
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 20-µL rxn.	Final Concentration
AccuStart Genotyping ToughMix, ROX (2X)	10 µL	1x
Forward primer	variable	100 – 900 nM
Reverse primer	variable	100 – 900 nM
Probe	variable	100 – 250 nM
Nuclease-free water	variable	
Template	2 – 5 µL	variable
Final Volume (µL)	20 µL	

Note: For smaller or larger reaction volumes scale all components proportionally.

PCR Cycling Protocol

PCR amplification can be carried out in a Real-Time PCR System or a conventional stand-alone Thermal Cycler. After PCR, fluorescent signal for each probe can be measured using a suitable fluorescent plate reader or Real-Time PCR System. Consult the operations manual for your instrument and/or choice of detection chemistry for appropriate PCR cycling conditions and data analysis instructions.

	Fast 2-Step Cycling	Fast 3-Step Cycling	Standard Cycling
Initial denaturation:	95ºC, 5 to 10 min *	95ºC, 5 to 10 min *	95ºC, 5-10 min *
PCR cycling (30-45 cycles):	95ºC, 3 to 5s	95ºC, 3 to 5s	95ºC, 10 to 15s
		55 to 65ºC, 15s	
	60ºC, 20 to 30s †	68 to 72ºC, 10s †	60ºC, 30 to 60s †

The appropriate step for fluorescent data collection varies for different probe assay formats. Data collection for 5'-nuclease probe assays (TaqMan probe) should be carried out at the end of the extension step. Use the annealing step for data collection with hybridization probe assays (HybProbe® FRET hybridization probes, Molecular Beacons, Solaris® qPCR Assays, Scorpions® primers, etc.).
End-point analysis should be carried out at a suitable temperature for your detection probe chemistry.

* Full activation of the DNA polymerase occurs within 10 seconds at 95ºC; however, optimal initial denaturation time is *template dependent* and will affect PCR efficiency and sensitivity. Amplification of genomic DNA or supercoiled plasmid DNA targets may require 5 to 10 min at 95ºC to fully denature and fragment the template. Short double-stranded DNA template (PCR product) or single-stranded DNA template, such as cDNA, may require as little as 1s at 95ºC. Use 30s at 95ºC as a general starting point.

† Extension time is dependent upon amplicon length and the minimal data collection time requirement for your qPCR instrument. Use 30s at 60ºC as a general starting point. Some assay designs and/or detection chemistries may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature and time may need to be empirically determined for any given primer set and real-time instrument.

Quality Control

Kit components are free of contaminating DNase and RNase. AccuStart Genotyping ToughMix, ROX is functionally tested for discrimination of a known SNP using a controlled gDNA sample panel, as well as for qPCR of a single copy gene in human gDNA. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($R^2 > 0.990$) with a 2-fold discrimination of starting template and a PCR efficiency > 95%.

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