PerfeCTa® FastMix® II

Cat No.	95118-250	Size:	250 x 20-µL reactions (2 x 1.25 mL)	**Store at -25ºC to - 15°C protected from light**
	95118-012		1250 x 20-µL reactions (10 x 1.25 mL)	
	95118-05K		5000 x 20-µL reactions (1 x 50 mL)	

Description

PerfeCTa FastMix II is an advanced qPCR reagent system for both fast and conventional PCR cycling protocols or instruments. It is a versatile and robust solution that provides the ultimate sensitivity and high PCR efficiency using a variety of fluorogenic probe chemistries, including TaqMan® hydrolysis probes. PerfeCTa FastMix II is provided as a 2X concentrated ready-to-use reaction cocktail that contains all required reaction components, except primers, probe(s), 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 qPCR performance or affect the stability of the product.

A key component of PerfeCTa FastMix II is an ultra pure, processive thermostable DNA polymerase that is free of detectable *E. coli* DNA§. PerfeCTa FastMix II is ideal for demanding qPCR applications such as bacterial pathogen detection where residual host DNA in typical recombinant enzyme preparations can limit assay sensitivity and obscure detection of low copy samples. The enzyme in PerfeCTa FastMix II is combined with high avidity monoclonal antibodies to provide a stringent automatic hot-start that allows reaction assembly, and temporary storage, at room temperature prior to PCR amplification.

Instrument Compatibility

Different real-time PCR systems 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. PerfeCTa FastMix II does ***not*** contain an internal reference dye. Please consult our Product Finder selection tool at www.quantabio.com to find the correct product for your real-time PCR system.

Components

PerfeCTa FastMix II, (2X):	2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, 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.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for qPCR:

- The design of highly specific primers and probes is a critical parameter for successful real-time 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 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.
- Suggested input quantities of template are: cDNA corresponding to 1 pg to 100 ng of total RNA; 10 pg to 1 µg genomic DNA
- 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
PerfeCTa FastMix II (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

	Fast 2-Step Cycling	Fast 3-Step Cycling	Standard Cycling
Initial denaturation:	95ºC, 30s *	95ºC, 30s *	95ºC, 2-3 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 qPCR 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. PerfeCTa FastMix II is functionally tested in qPCR. 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%. § Testing of bulk enzyme for residual *E. coli* genomic DNA is validated to be less than 1 copy / unit.

Limited Label Licenses

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