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Product Information

Fast Probe Master Mix

Catalog Number:
Without ROX: 31005-T, 31005, 31005-1, 31005-2
With ROX: 31016-T, 31016, 31016-1, 31016-2

Kit catalog numbers	1 x 1 mL 100 x 20 uL reactions	2 x 1 mL 200 x 20 uL reactions	5 x 1 mL 500 x 20 uL reactions	50 x 1 mL 5000 x 20 uL reactions
Fast Probe Master Mix (no ROX)	31005-T	31005	31005-1	31005-2
Fast Probe Master Mix (with ROX)	31016-T	31016	31016-1	31016-2

Components:
The product is 2X probe master mix that contains dNTP, optimized buffer and stablizer (including Tris and $MgCl_2$), and Cheetah™ HotStart Taq polymerase. Fast Probe Master Mix (with ROX) containing ROX reference dye, which may be required on ABI and certain other instruments (see master mix selection guide for real-time instruments below).

Storage and Handling
Fast Probe Master Mix is shipped on blue ice and should be stored immediately upon arrival at -20 °C. When stored in a constant temperature freezer at -20 °C, the kit is stable for at least 1 year from the date of receipt. Before use, thaw at room temperature and mix thouroghly by gentle vortexing. After thawing, the master mix should be kept on ice before use. It can be refrozen for storage.

Product Description
Fast Probe Master Mix is a ready-to-use hot-start mix for quantitative real-time analysis of DNA samples from various source. This product is suitable for all fluorescent probe-based technologies, including hydrolysis probes (such as TaqMan® and dual-Labeled BHQ® probes) and displacement probes (like molecular beacons).

This specially formulated probe master mix offers strong signal and high sensitivity, which make gene quantitation and viral load assessment possible even for the most challenging samples such as tissue, blood or serum. The wide range of reporter dye choices enable multi-gene detection in a single tube and provide reliable results for multiplex applications such as SNP gene typing and pathogen detection.

Although the master mix is formulated for qPCR using a fast cycling protocol, it is also compatible with qPCR using a regular cycling protocol (see below for recommended cycling protocols of Two-Step fast, Univeral and Three-Step regular). ROX reference dye is not required for most of non-ABI instruments and is not included in Cat #31005. For well-to-well fluorescence normalization in ABI and some other instrument platforms, use Cat #31016, to which ROX reference dye is conveniently included. Please refer to the master mix selection guide below to decide which master mix is best suited for your real-time PCR instrument.

Our master mixes include Cheetah™ Taq, our proprietary chemically-modified hot-start DNA Polymerase. Unlike AmpliTaq Gold®, which is also a chemically modified Taq but takes 10 minutes or longer to activate, Cheetah™ Taq is fully recovered in 2 minutes with high activity, making it particularly suitable for fast PCR. Cheetah™ Taq is completely inactive at room temperature and largely free of DNA contamination. This makes Cheetah™ Taq superior to any antibody-based hotstart Taq, which is typically not completely inactive at room temperature and is prone to DNA contamination due to the nature of antibody production.

This kit is suitable for mRNA quantitation if a two-step procedure is followed. The first step involves converting the mRNA to cDNA by reverse transcription (components not provided). A portion of the synthesized cDNA can then be quantitated by using Fast Probe Master Mix in the second step. To ensure optimal amplification efficiency, the aliquot of the cDNA sample to be amplified should not exceed 10% of the volume of the PCR reaction. For accurate quantitation of mRNA level, a none-RT control is recommended to eliminate the possibility of genomic DNA contamination.

For one-step RT-qPCR, reverse transcriptase (components not provided) is required. It is critical to have well characterized primer set that does not form primer-dimer. We recommend to titrate the amount of reverse transcriptase and the duration of the RT step. If possible, design primers to have Tm at 55 °C, run both RT step and extension step at 55 °C. For accurate quantitation of mRNA level, a none-RT control is recommended to eliminate the possibility of genomic DNA contamination.

References
1. Xin X and Mao F. AllGlo™ Probe: Highly Specific Homo-Labeled Probe Technology for Real-Time qPCR. 3rd International qPCR Symposium. 26th - 30th March 2007, in Freising-Weihenstephan, ISBN-13: 978-3-00-020385-5

2. Xin X. A closed-tube real time PCR system for simultaneous pathogen detection and mutation scanning. 2008. 11th WPCCID | PS3-078

3. Mao F, Leung WY, Xin X. Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. BMC Biotechnol. 2007 Nov 9;7:76.

Additional Notes:

1) Data analysis: Absolute quantification is performed by plotting samples of unknown concentration to a standard curve from a dilution series of template DNA of known concentrations. A standard curve is first generated by plotting Ct (y-axis, threshold cycle) against the logarithm of DNA amount (x-axis, copy number). A linear regression is used to calculate the amount DNA of unknown samples. The slope of this line represents the efficiency of PCR reaction, which should be -3.3 if the efficiency is 100%.

2) ROX reference dye: Fast Probe Master Mix with ROX (Cat #31016) contains ROX reference passive dye, which is required on ABI and some other instruments. If you wish to use a fluorescent probe with wavelength equivalent to ROX, please choose Fast Probe Master Mix (without ROX) (Cat # 31005).

   The concentration of ROX in a reaction will artificially affect the apparent read out of the fluorescence signals, but it will not affect the relative Ct as long as all reactions contain the same concentration of ROX. In general ABI 7900 needs more ROX reference dye than ABI 7500. Thermal Cyclers such as iCycler iQ5, iCycler IQ, MJ Opticon, MJ Chromo 4, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or LightCycler 480 do not need ROX reference dye for normalization.

3) Hydrolysis probes such as TaqMan® probe cannot be used for melt curve analysis. To take advantage of both probe specificity and melt curve capability, we recommend a multiplex PCR reaction with a probe and EvaGreen® dsDNA binding dye in a single tube (see reference 2.).

PCR Protocols

1. Reaction Setup

Pipet reaction components into each well according to the table below:

Reaction component	Amount required for a 20 uL reaction	Final concentration
2X Fast Probe Master Mix	10 uL	1X
Primers	x uL each	0.1-0.5 uM each
Probe	x uL each	0.1-0.5 uM each
Template	x uL	See Helpful Tip # 1
ROX	Optional	See Helpful Tip # 2
H_2O	Add to 20 uL	

2. Cycling Protocol

You may choose one of the following three protocols, depending on the nature of your amplicon and instrument capability.

A. Two-step fast cycling protocol

This cycling protocol should be applicable to hydrolysis probes when the amplification is short and the primer and probe T_m's are close to 60 °C.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	96 °C	2 min	1
Denaturation	96 °C	5 s (See Helpful Tip #3)	40
Annealing & Extension	60 °C	30 s	

B. Universal cycling protocol

This traditional cycling protocol can be used on nearly all qPCR instruments. The protocol may also benefit targets that are relatively difficult to amplify under fast cycling condition.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95 °C	5 min	1
Denaturation	95 °C	15 s	40
Annealing & Extension	50-60 °C (See Helpful Tip #4)	60 s	

C. Three-step cycling protocol

This cycling protocol can be used for probe systems like molecular beacons when Tm's of the probe is equal to or lower than 60 °C. An extra extension step at 72 °C helps the polymerase to completely extend the product.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95 °C	5 min	1
Denaturation	95 °C	15 s	40
Annealing	50-60 °C (Tip #4)	30 s	
Extension	72 °C (Tip #5)	30 s	

Helpful Tips:

1) Amplicon length: To maximize amplification efficiency with Fast Probe Master Mix, the optimal amplicon length is 50-100 bp. If longer amplicon is intended, you may need to extend elongation time. For hydrolysis probes which require 5' exonuclease activity, the close the probe to the primer, the high the signal the reaction generates.

2) ROX reference dye: ROX reference dye is not required for most of non-ABI instruments and not supplied in the mix (Cat # 31005). For well-to-well fluorescence normalization in ABI and some other instrument platforms, use the mix with ROX (Cat #31016), which ROX reference dye is conveniently included. Please refer to the master mix selection guide in page 2 to decide which master mix is best suited for real time instruments.

3) Denaturation time: The holding time for denaturation can be lower than 5 seconds, including as low as 0 second, if you have a relatively short amplicon. When the denaturation time is set to 0 second in the program, it merely means that the temperature will ramp to 96 °C and then will immediately ramp down with no delay. Setting the time to 5 s will ensures a more robust denaturation for relatively long or high GC amplicons. Instruments with fast ramping capability further add reliability to amplicon denaturation.

4) Annealing & Extension temperature: The Tm's of primers and probe can be designed identical and as low as 50 °C. Accordingly, the annealing & extension temperature should be set to match the Tm of the primers. This will benefit AT-rich amplicons.

5) Extension temperature: Taq extends most efficiently at 72 °C. However, for AT-rich amplicons (>70% AT) or amplicons that have an AT-rich patch, extension at 72 °C should be avoided.

Master Mix Selection Guide

Master Mix	PCR Instrument
Fast Probe Master Mix (without ROX) Cat# 31005	BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX-96 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000 & 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCyler® Roche: LightCycler® 480, LightCycler® 2.0
Fast Probe Master Mix (with ROX) Cat# 31016	ABI (Applied Biosystems®): 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™ 7500, 7500 Fast, ViiA™7, QuantStudio™ instruments Stratagene (Agilent): MX4000P, MX3000P, MX3005P

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

Related Products

Catalog number	Product
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)
31045	Forget-Me-Not™ EvaGreen® qPCR Master Mix (Low ROX)
31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix (High ROX)
31000	EvaGreen® Dye, 20X in water
29050	Cheetah™ HotStart Taq DNA Polymerase
29054	HotStart Polymerase Modification Kit
29051	EvaEZ™ Fluorometric Polymerase Activity Assay Kit
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in water
31042	Ready-to-Use 100 bp DNA Ladder
31022	Ready-to-Use 1 kb DNA Ladder
41042-4L	Water, Ultrapure Molecular Biology Grade
41006	TBE Buffer, 5X
E90003	Gel-Bright™ LED Gel Illuminator