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Product Information

Fast Probe Master Mix

Catalog Number: 31005, 31005-1, 31005-2 or 31005-T

Unit Size: 200 reactions (cat # 31005), 500 reactions (cat # 31005-1), 5,000 reactions (cat# 31005-2), or 100 reactions (cat # 31005-T, trial size)

Components:

The product is 2X probe master mix that contains dNTP, optimized buffer and stabilizer (including Tris and MgCl₂), and Cheetah™ hot-start Taq polymerase.

Cat #	Unit Size
31005	200 rxn (2 X 1 mL)
31005-1	500 rxn (5 X 1 mL)
31005-2	5,000 rxn (50 X 1 mL)
31005-T	100 rxn (1 X 1 mL)

Fast Probe Master Mix with ROX

Catalog Number: 31016, 31016-1, 31016-2 or 31016-T

Unit Size: 200 reactions (cat # 31016), 500 reactions (cat # 31016-1), 5,000 reactions (cat# 31016-2), or 100 reactions (cat # 31016-T, trial size)

Components:

The product is 2X probe master mix that contains dNTP, optimized buffer and stabilizer (including Tris and MgCl₂), Cheetah™ hot-start Taq polymerase and ROX reference dye, which may be required on ABI and certain other instruments (See master mix selection guide for real time instruments below).

This product is suitable for all color probes qPCR detection except for ROX channel probe. If you use ROX equivalent wavelength dye labeled probes (URA as in AllGlo probe), please choose Fast Probe Master Mix (Cat # 31005) instead.

Cat #	Unit Size
31016	200 rxn (2 X 1 mL)
31016-1	500 rxn (5 X 1 mL)
31016-2	5,000 rxn (50 X 1 mL)
31016-T	100 rxn (1 X 1 mL)

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 6 months from the date of receipt. Before use, thaw at room temperature and mix thoroughly 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 sources using probe-based detection. Although optimized for AllGlo™ probe system, this master mix is also suitable for other probe systems such as hydrolysis probes such as TaqMan® and Molecular Beacon®.

The AllGlo™ probe is a simple fluorogenic probe without quencher or MGB. AllGlo™ probes generate two light-emitting labels per probe, giving more intense fluorescence than traditional Taqman® probes. AllGlo™ probes are available in six colors, MAR, JUP, SAT, URA, NEP and PLU, which match with the absorption and emission wavelength of FAM, HEX, TAMRA, ROX, Cy5 and Quasar 670, respectively. These six AllGlo™ dye labels are compatible with most real time PCR instruments and generally require no special calibration. For detailed probe design guidelines refer to AllGlo™ user's manual (www.alleglogic.com).

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, Universal and Three-Step regular). 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 below to decide which master mix is best suited for real time instruments.

Another critical component of the master mix is 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 T_m 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 AllGlo Probe Master Mix with ROX (Cat #31016) contains optimal ROX reference passive dye, which is required on ABI and some other instruments. If you use ROX equivalent wavelength dye labeled probes (URA as in AllGlo probe), please choose Fast Probe Master Mix (Cat # 31005) instead.

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 AllGlo probes and EvaGreen dsDNA binding dye in one single tube (see reference 2.)

Master Mix Selection Guide for Real Time Instruments

Cat#	Product	PCR Instrument
31005	Fast Probe Master Mix	BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCycler Roche: LightCycler 480, LightCycler 2.0
31016	Fast Probe Master Mix with ROX	ABI: 7500, 7500 Fast, 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus Stratagene: MX4000P, MX3000P, MX3005P

* AllGlo, MAR, JUP, SAT, URA, NEP, and PLU are trademarks of AlleLogic Biosciences Corporation.; AllGlo dyes and probes and their use are covered by US and international patents (U.S. Patent No. 7,667,024). ** TaqMan and Molecular Beacon is a registered trademark of Molecular Probes, Inc. *** Practicing real-time PCR may require a license from Roche or Applied Biosystems, Inc.

Related Products:

EvaGreen dye, 20X in H₂O, Cat # 31000
 Cheetah Hotstart Taq DNA polymerase, Cat # 29050
 Fast EvaGreen Master Mix, Cat # 31003
 Fast EvaGreen Master Mix with Low ROX, Cat # 31014
 Fast EvaGreen Master Mix with High ROX, Cat # 31015
 dNTP Set, 100mM, Cat #40052
 dNTP Mix, 25mM, Cat # 40053
 dNTP Mix, 10mM, Cat # 40054
 PMA for selective detection of live pathogens by PCR, Cat # 40013
 GelRed nucleic acid gel stain, 10,000X in H₂O, Cat # 41003
 GelGreen nucleic acid gel stain, 10,000X in H₂O, Cat # 41005

PCR Protocols

1. Reaction Setup

To prepare probe stock, refer to the probe amount listed on the Oligonucleotide Specification Sheet. For each 1 nmole of fluorescence labeled probe, add 100 µl dH₂O to make 10 µM stock, which can be used as 20X if the final probe concentration is 500 nM.

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

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

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 closer the probe to the primer, the higher 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.

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	

Helpful Tip:

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 ensure a more robust denaturation for relatively long or high GC amplicons. Instruments with fast ramping capability further add reliability to amplicon denaturation.

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	

Helpful Tip:

4) **Annealing & Extension temperature:** The T_m'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 T_m of the primers. This will benefit AT-rich amplicons.

C. Three-step cycling protocol

This cycling protocol can be used for probe systems like Molecular Beacons when T_m '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 (See Helpful Tip #4)	30 s	
Extension	72 °C (See Helpful Tip #5)	30 s	

Helpful Tips:

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.

3. Data Acquisition

Probe-based fluorescence data acquisition depends on the chemistry used. For AllGlo probe or TaqMan chemistry, please refer to the following table for the excitation and emission wavelengths for each dye label.

AllGlo Probe Dye Spectral Properties			
AllGlo Dye	Detection Channel	Excitation Peak (nm)	Emission Peak (nm)
MAR	FAM	495	520
JUP	HEX/JOE/VIC	525	550
SAT	TAMRA	555	580
URA	ROX/TEXAS RED	575	600
NEP	Cy 5	635	670
PLU	Quasar 670/Alexa 680	685	720