Protocol

TD-P Revision 2.0

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Goof-Proof™ Universal Probe Protocol

Procedure for using Goof-Proof Universal Probe Master Mix

Introduction

Goof-Proof™ Universal Probe Master Mix is a high-performance 2X master mix for fluorescent probe-based PCR applications such as SNP genotyping and quantitation. Goof-Proof™ has broad instrument compatibility, can be used in both standard and fast protocols and shows a high degree of consistency in both singleplex and multiplex reactions. The Goof-Proof™ Universal Probe Master Mix is perfectly designed for all fluorescent probe technologies, including displacement probes such as Molecular Beacon and hydrolysis probes such as TaqMan®.

GoldBio's HotStart Taq DNA Polymerase is a proprietary chemically modified hot-start DNA polymerase. Our HotStart Taq is fully activated in 2 minutes with high activity recovery, making it particularly suitable for fast PCR and is completely inactive at room temperature.

The 40X Tracking Buffer contains an inert blue dye. You have the choice of adding Tracking Buffer to the master mix, to the DNA template, or not to use the tracking buffer in your reactions. Addition of Tracking Buffer to your master mix allows the user to easily distinguish wells containing reaction mix from empty wells. On the other hand, adding Tracking Buffer to your DNA template samples allows you to track which reactions have had template added while you set up your PCR reactions.

Kit Components

Component	G-710-1	G-710-5	G-715-1	G-715-5
Goof-Proof™ Universal Probe Master Mix	1 ml	5 x 1 ml	1 ml	5 x 1 ml
40X Template Buffer	1 ml	2 x 1 ml	1 ml	2 x 1 ml
ROX Reference Dye	n/a	n/a	0.2 ml	0.2 ml

Storage/Handling

Store the kit at -20°C. The Goof-Proof™ Universal Probe Master Mix is shipped on blue ice and should be stored immediately upon arrival at -20°C. When stored under the recommended condition and handled correctly, the kit should be stable for at least 1 year from the date of receipt. Before use, thaw at room temperature and mix well by gentle vortexing. It can be refrozen for storage.

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Figure 1. Reaction after adding template with 1X Goof-Proof™ Tracking Buffer

Method

PCR Reaction Mix

Reaction Component	Amount required per 20 µl reaction (1)	Final Concentration
2X Goof-Proof™ Master Mix	10 µl	1X
Primers	x µl each	0.1 - 0.9µM each
Fluorogenic probe	x µl	0.1 - 0.5µM
40X Tracking Buffer	Optional (0 - 0.5 µl)	See Notes
ROX	Optional (0 - 0.3 µl)	See Notes
Template	x µl (See Note 1)	See Notes
H2O	Add to 20 µl	

Note: Reaction volumes may be between 5 - 25 µl.

Note: The optimal primer and probe concentrations should be determined empirically; however, primer concentrations of 200 - 400nM and probe concentrations of 100 - 200nM are generally suitable for most applications.

Note: Tracking Buffer is optional. If you choose to add Tracking Buffer to the master mix, 50 µl can be added directly to 1 mL of 2X Goof-Proof™ Universal Probe Master Mix, or add 0.5 µl per 20 µl reaction when setting up the assay master mix. Tracking Buffer may be added to your template samples at a final reaction concentration of 1X. For example, dilute 40X Tracking Buffer to 20X in PCR grade water. Make 1:1 dilution of template with the 20X Tracking Buffer and add 2 µl of the mixture to the reaction.

Note: ROX is optional for some PCR instruments, and is required by other instruments as a passive reference dye to normalize small well to well detection differences. Refer to Table 1 for the recommended ROX concentration for your instrument. Do not use ROX when using orange fluorescent probes (e.g. JUN®, Texas Red®) as these probes are detected in the same channel as ROX.

Note: Template concentration. The optimal amount of template DNA varies by application. Recommended amounts of genomic DNA template per reaction typically range from 50 pg to 500 ng per reaction. Recommended amounts of cDNA typically range from 100 fg to 100 ng.

PCR Cycling Protocols

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 most amplifications where the primer T_m are designed to be 58-60°C.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95°C	2 minutes	1
Denaturation	95°C	2-5 seconds	40
Annealing & Extension	60°C	20-30 seconds	

B. Three-step fast cycling protocol

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95°C	2 minutes	1
Denaturation	95°C	3-5 seconds	40
Annealing	50-65°C	5 seconds	
Extension	72°C	20-25 seconds	

C. Universal cycling protocol

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

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95°C	2 minutes	1
Denaturation	95°C	10-15 seconds	40
Annealing & Extension	60°C	60 seconds	

Tips

PCR instruments. For Roche LightCycler users using glass capillaries for reactions, you need to add BSA to your PCR reactions (~0.5 mg/ml final concentration). BSA is not necessary if transparent plastic capillary tubes are used.

Table 6. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended ROX Concentration	Amount of ROX per 20 µl reaction
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96 Touch, CFX-384 Touch, Chromo4, MiniOpticon Qiagen: Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCyler Roche: LightCycler 480, LightCycler 2.0	No ROX	None
ABI: 7500, 7500 Fast, ViiA 7, QuantStudio Stratagene: MX4000P, MX3000P, MX3005P	Low ROX	If using Tracking Buffer, dilute ROX 1:100 with dH_2O and add 3 µl diluted ROX per 20 µl reaction. Or dilute ROX 1:10 with dH_2O and add 30 µl diluted ROX per 1 ml tube of master mix.
		If not using Tracking Buffer, dilute ROX 1:100 with dH_2O and add 2.5 µl diluted ROX per 20 µl reaction. Or dilute ROX 1:10 with dH_2O and add 25 µl diluted ROX per 1 ml tube of master mix.
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX	If using Tracking Buffer dilute ROX 1:10 with dH_2O and add 3 µl diluted ROX per 20 µl reaction. Or add 30 µl undiluted ROX per 1 ml tube of master mix.
		If not using Tracking Buffer, dilute ROX 1:10 with dH_2O and add 2.5 µl diluted ROX per 20 µl reaction. Or add 25 µl undiluted ROX per 1 ml tube of master mix.

Associated Products

GoldBio Catalog #	Product Name
A-201	Agarose LE (Molecular Biology Grade)
D010	1 kb DNA Ladder
D011	1 kb PLUS™ DNA Ladder
D001	100 bp DNA Ladder
E-670	EvaGreen® Dye, 20x (25µM) in Water
G-725	GelRed™ Nucleic Acid Stain Gel Stain, 10,000X in Water
G-745	GelGreen™ Nucleic Acid Stain Gel Stain, 10,000X in Water

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