Product Information

February 21, 2018

2X Forget-Me-Not™ Universal Probe qPCR Master Mix

Catalog Number
Without ROX: 31043-T, 31043-1
With separate tube of ROX: 31044-T, 31044-1

Unit Size:
31043-T, 31044-T: 1 mL (100 x 20 uL reactions)
31043-1, 31044-1: 5 mL (500 x 20 uL reactions)

Kit Contents

Component	31043-T	31043-1	31044-T	31044-1
2X Forget-Me-Not™ Universal Probe qPCR Master Mix (99813)	1 X 1 mL	5 X 1 mL	1 X 1 mL	5 X 1 mL
Forget-Me-Not™ 40X Tracking Buffer (99814)	1 X 1 mL	2 X 1 mL	1 X 1 mL	2 X 1 mL
ROX Reference Dye (31042C-200uL)	N/A	N/A	1 X 0.2 mL	1 X 0.2 mL

Storage and Handling
2X Forget-Me-Not™ Universal Probe qPCR Master Mix is shipped on blue ice and should be stored immediately upon arrival at -20°C. When stored as recommended 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. The kit components can be refrozen for storage.

Product Description
2X Forget-Me-Not™ Universal Probe qPCR Master Mix is a high-performance product for fluorescent probe-based PCR applications, including quantitation and SNP genotyping. This kit 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). Forget-Me-Not™ Universal Probe Master Mix shows excellent concordance of results in singleplex and multiplex reactions, and has broad instrument compatibility, in both standard and fast protocols.

The master mix contains Cheetah™ HotStart Taq DNA Polymerase and dNTPs in a buffer optimized for high qPCR sensitivity and multiplex reactions. Only primers, probe and template need to be added. The master mix can be used with or without ROX dye, and with or without Forget-me-Not™ Tracking Buffer.

The 40X Forget-Me-Not™ 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.

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

PCR Protocol

Prepare the PCR master mix

1) Thaw frozen kit and other reaction components, mix thoroughly, and store on ice. Protect ROX and fluorogenic probes from light.
2) Prepare an assay master mix of appropriate total volume for all reactions by combining reaction components (except template) as recommended in the table below. This can be done on ice or at room temperature.

Reaction Setup

Reaction component	Per 20 uL reaction [a]	Final concentration
2X Forget-Me-Not™ Universal Probe qPCR Master Mix	10 uL	1X
Forward and reverse primers	Variable	100-900 nM each [b]
Fluorogenic probe	Variable	100-500 nM [b]
ROX Reference dye	Optional (0 - 3 uL)	See note [c]
40X Tracking Buffer	Optional (0 - 0.5 uL)	See note [d]
Template	Variable	See note [e]
dH_2O	Add to 20 uL total	N/A

[a] Reaction volumes may be between 5 - 25 uL.

[b] Primers and probes should be designed using programs such as Primer3 (http://primer3.sourceforge.net/) or Primer Express® (Applied Biosystems). The optimal primer and probe concentrations should be determined empirically; however, primer concentrations of 200 - 400 nM and probe concentrations of 100 - 200 nM are generally suitable for most applications.

[c] 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.

[d] Tracking Buffer is optional. If you choose to add Tracking Buffer to the master mix, 50 uL can be added directly to 1 mL of 2X Forget-Me-Not Universal Probe Master Mix, or add 0.5 uL per 20 uL reaction when setting up the assay master mix. Or 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 uL of the mixture to the reaction.

[e] 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.

Note: Roche LightCycler® users using glass capillaries for reactions should add BSA to PCR reactions at ~0.5 mg/mL final concentration. BSA is not necessary if plastic capillary tubes are used.

Prepare the individual PCR reactions

1) Pipette the assay master mix and template into PCR tubes or PCR plate wells.
2) Close tubes, or seal the plate wells with clear adhesive film. Centrifuge briefly.
3) Place in PCR instrument, and start the PCR run protocol.

Cycling Protocols

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 most amplifications where the primer Tm's are designed to be 58 - 60 °C.

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

B. Three-step fast cycling protocol

This cycling protocol allows use of optimal annealing and extension temperatures.

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

C. Standard cycling protocol

Use this cycling protocol for qPCR instruments or master mixes that don't allow fast cycling. This protocol may be useful for targets that are difficult to amplify under fast cycling conditions.

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

Related Products

Catalog number	Product
31041	Forget-Me-Not™ qPCR Master Mix (without ROX)
31042	Forget-Me-Not™ qPCR Master Mix (with ROX)
29051	EvaEZ™ Fluorometric Polymerase Activity Assay Kit
31003	Fast EvaGreen® qPCR Master Mix
31000	EvaGreen® Dye, 20X in water
31005	Fast Probe Master Mix (no ROX)
31016	Fast Probe Master Mix (with ROX)
29050	Cheetah™ HotStart Taq DNA Polymerase
29054	HotStart Polymerase Modification Kit
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in water
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31027	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
40013	PMA dye
40069	PMAxx™, 20 mM in H_2O
E90002	PMA-Lite™ LED Photolysis Device

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.

Table 1. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended ROX Concentration	Amount of ROX per 20 uL reaction
BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX-96 Touch™, CFX-384 Touch™ and Connect™, 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 Required
ABI: 7500, 7500 Fast, ViiA 7™, QuantStudio™ Stratagene: MX4000P, MX3000P, MX3005P	Low ROX (~50 nM)	If not using Tracking Buffer, dilute ROX 1/100 with dH_2O and add 2.5 uL diluted ROX per 20 uL reaction. Or dilute ROX 1/10 with dH_2O and add 25 uL diluted ROX per 1 mL tube of master mix.
		If using Tracking Buffer, dilute ROX 1/100 with dH_2O and add 3 uL diluted ROX per 20 uL reaction. Or add 3 uL undiluted ROX per 1 mL tube of master mix.
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne®, StepOnePlus®	High ROX (~500 nM)	If not using Tracking Buffer, dilute ROX 1/10 with dH_2O and add 2.5 uL diluted ROX per 20 uL reaction. Or 25 uL of undiluted ROX per 1 mL tube of master mix.
		If using Tracking Buffer, dilute ROX 1/10 with dH_2O and add 3 uL diluted ROX per 20 uL reaction. Or 30 uL of undiluted ROX per 1 mL tube of master mix.