Revised: September 18, 2020

Product Information

N-Flux™ 5X Digital PCR Master Mix

Catalog Numbers: 31078-T, 31078

Kit Contents

Component	**31078-T** 50 X 20 uL reactions	**31078** 250 X 20 uL reactions
99857-200uL: N-Flux™ 5X Digital PCR Master Mix	1 X 200 uL	5 X 200 uL
99845-100uL: ROX Reference Dye, 40 uM	1 X 100 uL	1 X 100 uL

Storage and Handling

N-Flux™ 5X Digital PCR Master Mix is shipped on blue ice and should be stored at -20°C upon arrival. Store ROX component protected from light. When stored as recommended the product is stable for at least 1 year from the date of receipt.

Product Description

N-Flux™ 5X Digital PCR Master Mix is a high-performance master mix specially formulated to address the complexities of reagent flow through microfluidic chips for digital PCR assays and device development. This dye-free, probe-based master mix minimizes interactions with hydrophobic plastic surfaces, improves distribution of the sample into individual partitions, and maximizes end-point fluorescence for sensitive and accurate digital PCR. The kit contains master mix with Cheetah™ HotStart Taq DNA Polymerase and dNTPs in a specially formulated buffer and includes a separate vial of ROX passive reference dye for optional use.

N-Flux™ 5X Digital PCR Master Mix has been validated in both singleplex and multiplex probe-PCR reactions for genotyping and copy number variation analysis. The flexibility of the 5X concentration facilitates PCR with large sample volumes or samples that are too dilute for traditional 2X master mixes. N-Flux™ 5X Digital PCR Master Mix is dye-free and suitable for all fluorescent probe-based technologies, including hydrolysis probes (such as TaqMan® and dual-labeled BHQ® probes) and displacement probes (like molecular beacons). Only primers, probe, and template need to be added.

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.

General Considerations

- Thaw frozen kit and other reaction components, gently vortex to mix thoroughly, and store on ice. Kit components may be stored at 4°C to avoid freeze/thaws during the workflow. Protect ROX and fluorogenic probes from light.
- For multiplex reactions, first run reactions in singleplex to ensure that primer and probe sequences are sufficient for amplification. Probe fluorophore must match the optics of your instrument. Real-time PCR reaction conditions (Table 1) and cycling protocols (page 2) may be used for verification of primer and probes using qPCR.
- Prepare an assay master mix of appropriate total volume for all reactions, with or without ROX, according to documentation provided by manufacturer. Setup can be performed on ice or at room temperature.

Table 1: Real-Time PCR Reaction Setup

Reaction component	Per 20 uL reaction	Final Concentration
N-Flux™ 5X Digital PCR Master Mix	4 uL	1X
Forward and reverse primers	Variable	100 - 900 nM each[a]
Fluorogenic probe	Variable	100 - 500 nM each[a]
ROX Reference dye, 40 uM	Variable	See note[b]
Template	Variable	See note
dH_2O	Add to 20 uL total	N/A

[a] 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

[b] 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 2 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.

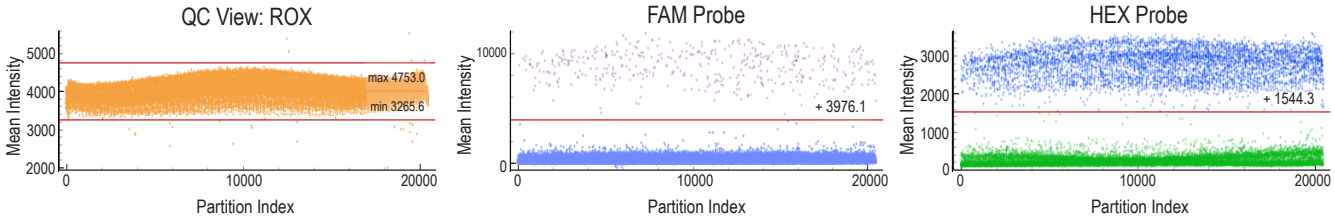

Figure 1. High-resolution, multiplex copy number variation analysis performed using N-Flux™ 5X Digital PCR Master Mix on the COMBiNATi Microfluidic Array Partitioning (MAP16) device. Superior partitioning results in quantitative determination of the copy number of a low abundance SNP variant and a high abundance control on human gDNA, with excellent separation between positive and negative wells.

Table 2. 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)	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 13 uL diluted ROX per 200 uL tube of master mix.
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne®, StepOnePlus®	High ROX (~500 nM)	Dilute ROX 1/10 with dH_2O and add 2.5 uL diluted ROX per 20 uL reaction. Or add 13 uL undiluted ROX per 200 uL tube of master mix.

Real-Time PCR Cycling Protocols

Choice of cycling protocol depends on your instrument capability and on the nature of your amplicon. If your instrument does not support fast cycling, use the parameters recommended in your instrument manual.

A. Two-step fast cycling protocol
This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60°C.

Cycling Step	Temperature	Holding Time	Number of cycles
Enzyme activation	95°C	2 min	1
Denaturation	95°C	2-5 sec	40
Annealing / extension / data acquisition	60°C	20-30 sec	

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	2-5 sec	40
Annealing	55-65°C	10 sec	
Extension / data acquisition	72°C	10-20 sec	

Related Products

Catalog number	Product
31077	EvaGreen® Plus Dye, 20X in water
31000	EvaGreen® Dye, 20X in water
29050	Cheetah™ HotStart Taq DNA Polymerase
29054	HotStart Polymerase Modification Kit
29051	EvaEZ™ Fluorometric Polymerase Activity Assay Kit
CD201	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD202	DNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix, (2-Color Tracking)
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
31022	Ready-to-Use 1 kb DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
41024-4L	Water, Ultrapure Molecular Biology Grade (4 L Cubitainer®)
41006	TBE Buffer, 5X (4 L Cubitainer®)
E90003	Gel-Bright™ LED Gel Illuminator

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

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