qPCR Master Mix Kits

For probe-based assays and arrays

Catalog #07516	1 Kit
Catalog #07517	1 Kit

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Product Description

qPCR Master Mix is a 2X concentrated solution optimized for TaqMan® probe-based, real-time quantitative polymerase chain reaction (qPCR). It is intended for use in combination with gene-specific primers (to amplify target cDNA) and fluorogenic-labeled probes that use the 5' nuclease activity of Taq DNA polymerase to produce a fluorescent signal. The rate of accumulation of the fluorescent signal is used to quantify the cDNA, and thereby to determine the amount of mRNA present in the original sample. qPCR Master Mix includes a Hot Start DNA polymerase, dNTPs, $MgCl_2$, enhancers, and stabilizers. The qPCR Master Mix Kit also includes ROX Reference Dye as a separate component, making it compatible with both reference dye-dependent and -independent qPCR systems.

- Efficient, sensitive, and reproducible
- Optimal performance when using standard or fast cycling conditions
- Ideal for high-throughput applications and overnight experiments
- Compatible with various real-time qPCR instruments

Product Information

The following components are sold as a complete kit (Catalog #07516 or 07517) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
qPCR Master Mix Kit (#07516)				
qPCR Master Mix	07509	1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
ROX Reference Dye	07519	200 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
qPCR Master Mix Kit (#07517)				
qPCR Master Mix	07510	5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
ROX Reference Dye	07519	200 µL	Store at -20°C.	Stable until expiry date (EXP) on label.

Components may be shipped at room temperature (15 - 25°C) but should be stored at -20°C as indicated above.

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

A. PREPARATION OF REACTION MIX AND qPCR PLATE

1. Thaw qPCR Master Mix, primer and probe solutions, template DNA, and ROX Reference Dye (if using) on ice.
2. If using ROX Reference Dye, add to qPCR Master Mix according to Tables 1 and 2. For instruments not listed, refer to the manufacturer's instructions.

Table 1. Reference Dye Concentration Levels for PCR Systems

PCR SYSTEM	REFERENCE DYE CONCENTRATION LEVEL		
	HIGH	LOW	NONE
Applied Biosystems • 7900HT Fast and 7300 Real-Time PCR Systems • StepOne™ and StepOnePlus™ Real-Time PCR Systems	X		
Applied Biosystems • ViiA™ 7 and 7500 Real-Time PCR Systems • QuantStudio™ Flex		X	
Agilent Technologies • Mx3005P and Mx4000P		X	
Bio-Rad • CFX, iQ™, and DNA Engine Opticon® Real-Time PCR Systems			X
Roche • LightCycler® Real-Time PCR System			X

Table 2. Volume of ROX Reference Dye to Add to Master Mix

VOLUME OF MASTER MIX	VOLUME OF ROX REFERENCE DYE (µL)	
	High Reference Dye System (see Table 1)	**Low Reference Dye System** (see Table 1)
1 mL	40	4
5 mL	200	20

3. Swirl vial/bottle of qPCR Master Mix to mix thoroughly.
4. Prepare the reaction mix as outlined in Table 3. Gently vortex to mix thoroughly.

Table 3. Reaction Mix Components

COMPONENT	FINAL CONCENTRATION OR AMOUNT	VOLUME PER 10 µL REACTION VOLUME
qPCR Master Mix	1X	5 µL
Forward and reverse primers	250 - 1000 nM each	Variable
Probe(s)	150 - 250 nM each	Variable
DNA template or controls	3 pg - 100 ng	Variable
Nuclease-free water (e.g. Catalog #79001)	Variable	Bring to 10 µL

5. Add an equal volume of reaction mix to each well of the qPCR plate.
6. Seal the plate using optical adhesive film.
7. Centrifuge the sealed plate at 1000 x *g* for 1 minute at room temperature (15 - 25°C) to remove bubbles from the bottom of the wells.
   NOTE: Bubbles in the bottom of the wells will interfere with results.
8. Place the plate on ice.

B. qPCR

1. Program the thermocycler according to manufacturer's instructions.
2. If reference dye is being used, calibrate thermocycler.
3. Add plate and run PCR program.

Related Products

For related products, including qPCR arrays available for characterizing the gene expression profile of various stem and progenitor cells and their derivatives following in vitro differentiation, visit www.stemcell.com or contact us at techsupport@stemcell.com.