qPCR 2X GREEN Master Mix

Without ROX™

Cat #: 42-117PG

Content: 4000 reactions (25 µL)
40 x 1.25 ml

Storage: -20°C.

Reagent for *in vitro* laboratory use only

Key Features

- All-in-one optimized master mix, including green dye
- High sensitivity
- High efficiency and specificity
- Wide dynamic range
- High reproducibility
- Hot start capacity for room temperature setup

Detection limit: Approximately 1 copy.

Quantification limit: Approximately 24 copies (0.08 ng of human gDNA, correlating to 12 diploid genomes, with 2 gene copies per diploid genome)

Compatibility: Real-time instruments with no requirement for normalization to an internal reference dye.

Introduction

Quantitative PCR is an important tool for SNP and gene expression analysis. Two general fluorescent chemistries exist for quantitative detection of gene transcripts: probes (e.g. TaqMan®, Scorpions™ Probes, molecular beacons) and DNA-binding fluorescent dyes (e.g. ethidium bromide, SYBR® Green, EvaGreen®, PicoGreen®). Apex qPCR 2X Master Mix is offered in two formulations: for probe or with DNA-binding fluorescent dye, making them ideal for most quantitative PCR applications.

The qPCR 2x Master Mixes are available with high, low or without ROX™ for optimal performance on most of the commonly used real-time PCR instruments. The qPCR 2X Master Mixes promote high specificity and low background by using Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

The Apex 2X GREEN Master Mix, without ROX™, is a single-tube 2x reagent including all components necessary to perform DNA-binding dye based real-time DNA amplification. Just add your primers and DNA.

Composition of qPCR 2X GREEN Master Mix, Without ROX™:

- Hot Start DNA Polymerase
- Optimized buffer system including dNTPs and green dye

Storage and Stability

The unopened product is stable at -20 °C for 2 years.

Storage Conditions after Thawing

Store the qPCR 2X Master Mix at +4 °C after thawing. Once thawed, full activity is guaranteed for 3 months.

Quality Control

The Hot Start DNA Polymerase is tested for contaminating activities, with no trace of endonuclease activity, nicking activity or exonuclease activity. The qPCR 2X GREEN Master Mix, Without ROX™, is functionally tested for efficiency and absence of contaminating human genomic DNA.

Pre-protocol Considerations

PCR Primers

It is important - especially in fluorescent DNA dye based quantitative PCR applications - to minimize the formation of non-specific amplification products. Particularly at low target concentration it is important to use the lowest possible primer concentration without compromising the efficiency of the PCR. The optimal concentration of primer pairs is the lowest concentration that results in the lowest C_t and an adequate fluorescence for a given target concentration with minimal or no formation of primer-dimers. The optimal concentrations of upstream and downstream primers are not always of equal molarity. Optimal concentrations of primers are in the range of 50 nM to 600 nM.

Preventing Template Cross-Contamination

Due to the high sensitivity of quantitative PCR there is a risk of contaminating the reactions with the products of previous runs. To minimize this risk, tubes or plates containing reaction products should not be opened or analysed by gel electrophoresis in the same laboratory area used to set up reactions.

Protocol

Note:

- Prior to the experiment, it is crucial to carefully optimize experimental conditions and to include controls at every stage. See pre-protocol considerations for details.

- Thaw the qPCR 2X Master Mix. Following initial thawing of the master mix, store the unused portion at +4 °C.

  Important: Multiple freeze-thaw cycles should be avoided. Solutions containing fluorescent green DNA dye should be protected from light whenever possible.

1. Prepare the experimental reaction by adding the components in the order shown in table 2.

Table 2. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
Apex qPCR 2x Master Mix	12.5 µl	1x
Primer A (10 µM)	0.5 µl (0.5 – 5 µl)	0.4 µM (0.1 – 1.0 µM)**
Primer B (10 µM)	0.5 µl (0.5 – 5 µl)	0.4 µM (0.1 – 1.0 µM)**
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 20 ng (1 – 100 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	25 µl	-

* Suggested starting conditions; theoretically used conditions in brackets

** Optimization of primer and probe concentrations is highly recommended.

2. Gently mix without creating bubbles* (do not vortex).

* Bubbles interfere with detection of fluorescence.

3. Place the reaction in the instrument and run the appropriate program according to the manufacturer's instructions.

Three-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	15 minutes	95 °C
40	15 – 30 seconds[b] 30 seconds[c] 30 seconds	95 °C 55 – 60 °C[d] 72 °C

Two-step PCR Program (recommended)

Cycles	Duration of cycle	Temperature
1[a]	15 minutes	95 °C
40 - 50	15 – 30 seconds[b] 60 seconds[c]	95 °C 55 – 60 °C[d]

a. For activation of the Hot Start DNA Polymerase.

b. Denaturation time is varying between thermocyclers.

c. Set the real-time instrument to detect and report fluorescence during the annealing/extension step of each cycle.

d. Choose an appropriate annealing temperature for the primer set used.

Related Products

Taq Polymerase kits (500 units)	Cat#
With 10X Standard and Ammonium Reaction Buffer	42-800B1
With 10X Combination Buffer	42-800B3
Glycerol Free	42-800B4

Hot Start DNA Polymerase (500 units)	Cat#
With 10X Ammonium and Combination Reaction Buffer	42-106

High Fidelity - Proof reading (500 units)	Cat#
Hi-Fi PR™ Taq 2.5 U/µl	42-110

All polymerases are also available in kits, Mg^{2+} free buffers and 50 mM $MgCl_2$.

Master Mixes (500 reactions)	Cat#
2X Taq DNA Polymerase Master Mix, 1.5 mM $MgCl_2$	42-132
2X Taq RED Master Mix, 1.5 mM $MgCl_2$	42-138
2X Hot Start Master Mix Buffer I, 1.5 mM $MgCl_2$	42-198

The shown master mixes are ammonium based. Also available with balanced ammonium and potassium based buffers.

Real-time PCR (400 reactions)	Cat#
qPCR 2X Master Mix for Probe, without ROX™	42-116P
qPCR 2X Master Mix for Probe, low ROX™	42-118P
qPCR 2X Master Mix for Probe, high ROX™	42-120P
qPCR 2X GREEN Master Mix, without ROX™	42-116PG
qPCR 2X GREEN Master Mix, low ROX™	42-118PG
qPCR 2X GREEN Master Mix, high ROX™	42-120PG

Ultrapure dNTPs	Cat#
dNTP set, 100 mM each: 250 µl of each dA, dC, dG and dT	42-410
dNTP Set, 100 mM each: 1 ml of each dA, dC, dG and dT	42-403
dNTP Mix 40 mM (1 x 500 µl): 10 mM each dA, dC, dG, dT	42-411
dNTP Mix 100 mM (2 x 1 ml): 25 mM each dA, dC, dG, dT	42-405
dNTP Mix 10 mM (10 x 1 ml): 2.5 mM each dA, dC, dG, dT	42-406

Other concentrations and Single dNTPs are available.

DNA Ladders	Cat#
Apex 100 bp-Low DNA Ladder, 250 applications	19-109
Apex 1 kb DNA Ladder, 333 applications	19-115
Apex 200 bp DNA Ladder, 200 applications	19-111
Apex ECON Mini DNA Ladder, 100 applications	19-130
Apex ECON Low DNA Ladder, 100 applications	19-131
Apex ECON PCR Ladder, 100 applications	19-132

Accessory reagents	Cat#
50 mM $MgCl_2$, 3 × 1.5 ml	42-303
Nuclease-Free Water, PCR Grade, 6 x 5 ml	42-710