Apex™ Reagents and Chemicals
A Genesee Scientific Brand

*******SAMPLE PACK*******

qPCR 2X Master Mix for Probe

Low ROX™

Cat #: SMP42-118P

Content: 40 reactions (25 µL)
0.5 ml

Storage: -20°C.

Reagent for *in vitro* laboratory use only

Key Features

- All-in-one optimized 2x master mix, including ROX™
- Suitable for quantification
- Suitable for multiplexing
- High efficiency and sensitivity
- Wide dynamic range
- High reproducibility
- Hot start capacity for room temperature setup

Detection limit: Approximately 2 copies (~0.007 ng of human gDNA, correlating to 1 diploid genome, with 2 gene copies per diploid genome).

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 which require low ROX™ as internal reference dye e.g. the Stratagene MX3005P.

Introduction

The Apex qPCR 2X Master Mix for Probe, Low ROX™ is a single-tube 2x reagent including all components necessary to perform probe based real-time DNA amplification. The Apex qPCR 2X Master Mix for Probe, Low ROX™ is suitable for multiplexing for up to four DNA targets in the same tube, thereby saving PCR consumables, time, workload and valuable DNA. Just add your probes, primers and DNA.

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 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.

Composition of qPCR 2X Master Mix for Probe, Low ROX™:

- Hot Start DNA Polymerase
- Optimized buffer system including dNTPs

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 Master Mix for Probe, Low ROX™, is functionally tested for efficiency and absence of contaminating human genomic DNA.

Pre-protocol Considerations

PCR Primers and probes

The design of primers and probes is critical especially for successful multiplex real-time PCR

- Design primers with similar annealing temperature.
- Analyse primer and probe sequences to avoid primer/probe hairpins, homo- or heterodimers, or any primer/probe complementarity across the targets.
- Optimization of primer and probe concentrations is highly recommended.
- Test assay efficiency by running each assay in singleplex reactions before conducting multiplex qPCR.
- Choose reporter dyes with appropriate excitation wavelengths with little to no overlap in their emission spectra. Check the instrument manual for recommendations.

Amplicon size

Recommended amplicon size is less than 200 bp.

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.

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)	1 µl (0.25 – 2 µl)	0.4 µM (0.1 – 0.8 µM)**
Primer B (10 µM)	1 µl (0.25 – 2 µl)	0.4 µM (0.1 – 0.8 µM)**
Probe (10 µM)	0.625 µl (0.125 – 0.625 µl)	0.25 µM (0.05 – 0.25 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

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

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

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	42-132
2X Taq RED Master Mix	42-138
2X Hot Start Master Mix Buffer I	42-198
2X Hot Start Master Mix Buffer I Blue	42-144

The shown master mixes are ammonium based with 1.5 mM $MgCl_2$. Also available with balanced ammonium and potassium based buffers.

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
PCR Grade Water 6 x 5 ml	42-710