Ampliqon

RealQ-PCR dUTP-UNG Master Mixes Kit (7mM $MgCl_2$)

With dUTP and uracil-N-glycosylase (UNG enzyme)

(Final $MgCl_2$ is 3.5mM)

With-out Green DNA Dye I

For 200 Reactions of 50µl pr Reactions

Cat. No.: 250707

ROX dye already included in the 2x RealQ Master Mixes. No need to add ROX dye.

Cat. No.	Size Reactions	Kit
250403	200	RealQ-PCR Master Mix (3mM $MgCl_2$)
250406	200	RealQ-PCR Master Mix (6mM $MgCl_2$)
250407	200	RealQ-PCR Master Mix (7mM $MgCl_2$)
250410	200	RealQ-PCR Master Mix (10mM $MgCl_2$)
250503	200	RealQ-PCR Master Mix (3mM $MgCl_2$), *with Green DNA dye in mix*
250506	200	RealQ-PCR Master Mix (6mM $MgCl_2$), *with Green DNA dye in mix*
250507	200	RealQ-PCR Master Mix (7mM $MgCl_2$), *with Green DNA dye*
250510	200	RealQ-PCR Master Mix (10mM $MgCl_2$), *with Green DNA dye in mix*
250603	200	RealQ-PCR Master Mix (3mM $MgCl_2$), *with Green DNA dye in separate tube*
250606	200	RealQ-PCR Master Mix (6mM $MgCl_2$), *with Green DNA dye in separate tube*
250607	200	RealQ-PCR Master Mix (7mM $MgCl_2$), *with Green DNA dye in separate tube*
250610	200	RealQ-PCR Master Mix (10mM $MgCl_2$), *with Green DNA dye in separate tube*
250703	200	RealQ-PCR dUTP-UNG Master Mix (3mM $MgCl_2$)
250706	200	RealQ-PCR dUTP-UNG Master Mix (6mM $MgCl_2$)
250707	200	RealQ-PCR dUTP-UNG Master Mix (7mM $MgCl_2$)
250710	200	RealQ-PCR dUTP-UNG Master Mix (10mM $MgCl_2$)

Store at -20°C. *Reagent for in-vitro laboratory use only*

Important information for the user

This kit is intended for more experience users that needs high quality products to an affordable price. It is not the intention of this instruction insert to give a complete overview of the Quantitative PCR methods but simply a short guide describing the most important issues for running Quantitative PCR using RealQ-PCR products. For more detailed description please consult the original manuals coming with the Quantitative PCR Instrument.

Components already included in the 2x RealQ-PCR dUTP-UNG Master Mix: Optimized buffer system, ROX Reference dye, dATP, dCTP, dGTP, dUTP and uracil-N-glycosylase (HK-UNG).

The $MgCl_2$ concentration is 7mM, which gives an $MgCl_2$ concentration of 3.5mM in the final reaction.

Introduction

Quantitative PCR has become an important tool for SNP and gene expression analysis. Several different fluorescent chemistries exist for either detection of SNP or quantitative gene transcripts. The use of fluorescent probe technologies reduces the risk of sample contamination while maintaining convenience, speed and high throughput screening capabilities. Ampliqon has developed the RealQ-PCR dUTP-UNG Master Mix, a single-tube 2X reagent ideal for most Quantitative PCR applications. The RealQ-PCR dUTP-UNG kit support quantitative amplification and detection with multiplex capability. The RealQ-PCR dUTP-UNG kit has been designed for optimal performance on ABI PRISM™ Instruments, the LightCycler™ Instrument, the Mx4000™ Instrument and the DNA Engine Opticon™ System. The RealQ-PCR dUTP kits includes the components necessary for performing PCR amplification, and have been successfully used to amplify and detect a variety of DNA targets such as genomic DNA, cDNA and plasmid DNA.

The RealQ-PCR dUTP-UNG master mix includes the TEMPase Hot Start DNA polymerase, a modified Taq DNA polymerase with hot start capabilities. The TEMPase Hot Start enzyme improves the PCR amplification reaction by decreasing background from non-specific amplification and increases amplification of desired products.

Materials provided

Materials provided (per kit)	Quantity
2X RealQ PCR dUTP-UNG Master Mix (7mM $MgCl_2$)	4x 1.25 mL
50X Glass blocking agents (LightCycler™)	200 µL
$MgCl_2$ Concentration: 25 mM	1.5 mL

Storage Conditions

Upon receipt, store all components at –20°C.
Store the 2X master mix at + 4°C after thawing. Once thawed, full activity is guaranteed for 3 month. Glass blocking agents and $MgCl_2$ can be stored at both -20°C and +4°C.

PRE-PROTOCOL CONSIDERATIONS:

Preventing Template Cross-Contamination

Due to the high sensitivity of Real Time PCR it is a risk that reaction may be contaminated with the products of previous runs. To minimize this risk, tubes or plates containing reaction products should not be opened or analyzed by gel electrophoresis in the same laboratory area used to set up reactions. Both dUTP and uracil-N-glycosylase (HK-UNG) is included in the RealQ PCR dUTP-UNG master mix to prevent PCR products from becoming source of contamination.

PCR Primers

It is important especially in Green DNA dye I based Quantitative PCR applications to minimize the formation of non-specific amplification products. Especially at low target concentration it is important to use the lowest primer concentration without compromising the efficiency of PCR. The optimal concentration of primer pairs is the lowest concentration that results in the lowest Ct and an adequate fluorescence for a given target concentration with minimal or no formation of primer-dimer.
The optimal concentrations of upstream and downstream primers are not always of equal molarity.

Primer concentration	Primer concentration for use in Multiplex PCR
50 to 600 nM	20 to 200 nM

Primer concentration optimization scheme

Magnesium Chloride

The optimal $MgCl_2$ concentration gives maximal amplification of a specific target amplicon with minimal non-specific products and primer-dimer formation. It is important especially in Green DNA I dye based Quantitative applications to optimize the $MgCl_2$ level, to avoid detection of non-specific dsDNA including primer-dimers. In general the $MgCl_2$ concentration in Green DNA I dye based application should be between 1.5 and 5.0 mM. The master mix is supplied with a final $MgCl_2$ concentration of 3.5 mM. For adding extra $MgCl_2$ please consult the below table.

Final $MgCl_2$ conc. in reaction (mM)	3.5	4.0	4.5	5.0
Additional volume of 25 mM $MgCl_2$ per 50 µl reaction (µL):	0	1	2	3

$MgCl_2$ dilution scheme

Reference Dye

A passive reference dye is included in the 2x RealQ-PCR dUTP-UNG Master Mix kit to compensate for non-PCR related variations in the fluorescence. The fluorescence from the passive reference dye does not change during the course of the PCR reaction but provide a stable baseline to which samples are normalized. The excitation and emission of the reference dye are 584 nm and 612 nm, respectively.

Glass blocking agents (LightCycler™)

One extra challenge using the LightCycler™ instrument is that the PCR reagents can form precipitate on the glass capillary surface as the Real time PCR progresses. To prevent this event Ampliqon has designed a special reagent for blocking the glass capillaries during the Quantitative PCR reaction. The Glass blocking agent comes as a 50X solution (1 µl pr 50 µl PCR reaction).

Protocol

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

Thaw the 2x RealQ-PCR dUTP-UNG Master Mixes and store on ice. Following initial thawing of the master mix, store the unused portion at +4°C.

Note: Multiple freeze-thaw cycles should be avoided.

Prepare the experimental reaction by adding the components in the following order:

25 µl of 2X master mix
x µl of experimental probe (optimized concentration)
x µl of upstream primer (optimized concentration)
x µl of downstream primer (optimized concentration)

Gently mix the reactions without creating bubbles (do not vortex).

Add x µl of experimental gDNA, cDNA or plasmid DNA to each experimental reaction.

Add Nuclease-free PCR-grade H_2O to adjust the final volume to 50µl (including experimental DNA)

Gently mix the reaction without creating bubbles (do not vortex).

Note: Bubbles interfere with fluorescence detection.

Place the reaction in the instrument and run the appropriate program below.

2-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	2 minutes	50 °C
1[b]	15 minutes	95 °C
40	15-30 seconds[c]	95 °C
	1.0 minute[d]	55-60 °C[e]

[a] Can be excluded if UNG is not used.
[b] For activation of the TEMPase hot start enzyme.
Note: The uracil-N-glycosylase enzyme is inactivated after 3 min at 95°C.
[c] Varying between thermocycles, used 30 seconds for the ABI PRISM 7700 instrument.
[d] Set the temperature cycler to detect and report fluorescence during the annealing/extension step of each cycle.
[e] Choose an appropriate annealing temperature for the primer set used.

3-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	2 minutes	50 °C
1[b]	15 minutes	95 °C
40	30 seconds[c]	95 °C
	1.0 minute[d]	55-60 °C[e]
	30 seconds	72 °C

[a] Can be excluded if UNG is not used.
[b] For activation of the TEMPase hot start enzyme.
Note: The uracil-N-glycosylase enzyme is inactivated after 3 min at 95°C.
[c] Varying between thermocycles, used 30 seconds for the ABI PRISM 7700 instrument.
[d] Set the temperature cycler to detect and report fluorescence during the annealing/extension step of each cycle.
[e] Choose an appropriate annealing temperature for the primer set used.

Related Products

Description	Cat. No.
Taq DNA Polymerase (500 Units) *with 10X Ammonium Reaction Buffer* *with 10X Standard Reaction Buffer*	**110303**
Taq DNA Polymerase (500 Units) *with 10X Combination Buffer*	**110403**
Taq DNA Polymerase (500 Units) *with 10X Mg^{++} Free Ammonium Buffer*	**110503**
Taq DNA Polymerase 2.0X Master Mix (100 Reac) *with 2.0 mM MgCl2*	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 Reac) *with 1.5 mM MgCl2,*	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) *with 2.0 mM MgCl2*	**190301**
AccuPOL DNA Polymerase (500 Units)	**210303**
TEMPase Hot Start DNA Polymerase (500Units) *with 10X TEMPase Buffer I* *with 10X TEMPase Buffer II*	**220303**
UniPOL –Long Range PCR (100 Reac)	**270701**
Rapid Ligation Kit (50 React)	**750300**
RT-PCR One Tube (100 Reac)	**740301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer I* (100 Reac)	**230301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer II* (100 Reac)	**230701**
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	**501004**
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	**502004**
GC5 Value Efficiency, 10^8 Cfu/µg pUC19 Chemically Competent Cells, (10x 200µl)	**812010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (10x 50µl)	**805010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (5x 200µl)	**802005**
SuperPath GC10, 10^{10} Cfu/µg pUC19 ElectroCompetent Cells, (5x 80µl)	**830805**
SOC Medium, 10x 10mL	**800000**

NOTICE
In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.